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A NEW TUMOR OF THE APRICOT[1]

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INTRODUCTION

A peculiar disease manifesting itself in the formation of tumors, or ılls, on the trunk and limbs of old apricot trees was investigated by ıe writer in California in 1916. While, as will be seen, the findings of ıat year do not amount to a complete knowledge of the disease, it has ɛmed desirable, in view of the writer's inability to follow the work to s completion, to publish the results thus far obtained, so that they ıay constitute a starting point for some other worker who may desire) investigate further this interesting disease.

HISTORY AND DISTRIBUTION OF THE APRICOT GALL

The apricot gall was not reported by growers until it had been in xistence for a fairly long period of years. While nothing definite can e said concerning the date of its first appearance, it seems to have been nown in Santa Clara and Alameda Counties, Calif., for at least 15 years revious to these investigations. Its spread during those years was very low. A marked progress, however, was noted, so one grower informed he writer, during the spring following the unusually wet winter of 1915, hen as many as 60 trees became affected in an orchard where only 1 iseased tree had been known to exist for the 12 previous years. At resent the disease occurs in Alameda and Santa Clara Counties, especially round Hayward, San Jose, and Niles, extending also into San Benito ounty. In this region numerous cases have been observed both on olated trees and on groups of trees forming diseased spots in the rchards. Recently several cases have been reported from Santa ruz County, Calif., and apparently the same disease has been found on rench prunes in Napa County.

DESCRIPTION OF THE DISEASE

New galls usually make their appearance during the moister part of he spring, in March or early April. Their period of growth coincides vith the growing season of the tree. During their first season the galls lo not, as a rule, become very prominent. They form eruptions reaching

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some 2 or 3 inches in longitudinal and transverse dimensions, and do not extend beyond the normal diameter of the limb by more than ½ to 1 inch. The surface of such young galls is usually rough, fissured longitudinally, and but little cracked transversely. Several such young galls, on a seriously affected limb, may be seen in Plate 1, A. Plate 1, B, shows an old gall. Note that it is very prominent and deeply fissured. This is a feature which is characteristic of the older galls, and more especially of those encircling the limbs on which they are borne. Another feature which may be observed on encircled limbs is the constriction forming just above and below the galls. This may be noted on Plate 1, B, but more particularly on Plate 3, which shows the encircled limb shown in Plate 1, B, in a longitudinal section. The galls form principally on the limbs and the trunk, and occasionally, although rarely, on the crown and roots of the trees. Plate 2, A, shows the only gall found on the root system of a tree whose limbs had reached the far advanced stage of disease shown in Plate 2, B. On going through an affected spot in the orchard, galls of all sizes may be noticed, ranging from small eruptions 1 inch in diameter to highly prominent galls measuring 18 inches transversely, according to the age of the swellings.

Alarming as this disease may seem to the casual observer, the presence of these tumors does not seem to have a very detrimental effect upon the vitality or productivity of the trees. Trees with as many as 10 to 15 galls on them do not show any appreciable reduction in their seasonal growth or yield. Not until the galls have increased in number so as to become almost contiguous on the trunk or main limb (Pl. 2, B), does the tree begin to show any signs of reduced vitality. Such a condition rarely if ever obtains until after 10 or 12 years from the appearance of the first gall. Up to the present time no trees are known to have died of this disease; nevertheless it seems beyond doubt that the affected trees will, if left to themselves, die ultimately of the disease, or at least suffer such a reduction of vitality as to make them worthless.

Investigation has established the following facts: (1) The disease never occurs on young trees. All the affected trees are advanced in years, usually not much less than 25 to 30 years old. (2) Galls form only on fairly old wood of these trees. In no case have any galls been found on wood less than 7 to 10 years old, as estimated by the yearly pruning cuts. (3) The disease, on the apricot, is practically restricted to the Moorpark variety.

In Mr. T. C. Gorrie's orchard at Hayward, Calif., where most of the field work has been carried out, rows of Moorpark apricot trees are planted beside Blenheim apricots of the same age, with occasionally trees of the two varieties mixed in the rows. Of about 80 Moorpark trees, nearly 60 are affected with the disease, while only a single gall has been found among all the Blenheim trees in this 8-acre orchard. Although trees of other apricot varieties may become affected, no cases have come to the writer's knowledge.

GROSS ANATOMICAL FEATURES OF THE GALLS

The anatomy of the disease has been found very characteristic, and its main features have proved to be of great diagnostic value. Plate 3 shows a longitudinal section through a limb bearing a gall. An examination of the tumor, with regard to the tissues involved in its formation, brings out the following facts:

1. The wood in the region underlying the tumor growth is somewhat hypertrophied, as is seen from a comparison of the diameter of the wood in this region with its diameter at a point beyond the constrictions referred to above.

2. The line of demarcation between the wood and the bast is sharp. The wood does not extend into the region which constitutes the excrescence, properly speaking. This line of demarcation, which is perfectly definite in the specimen shown on Plate 3, is somewhat vague in the one in Plate 4, A, showing a longitudinal section of an affected root; but in neither case is the wood found mingled in the tissues composing the tumor.

This distinctive feature of the disease is more clearly brought out when the views of the longitudinal sections shown on Plates 3 and 4, A, are contrasted with the view in Plate 4, B, showing a limb with an "aerial crowngall" on it in longitudinal section. In the case of the gall caused by *Bacterium tumefaciens* it is evident that the tumor consists largely of wood tissue. In fact, the bark has not increased much in thickness, while the wood has been greatly hypertrophied. In the apricot disease, however, the excrescence consists primarily of tissues lying outside of the cambium line; the wood, which is but slightly hypertrophied, having failed to extend into the tumor. This apricot disease is therefore a bark excrescence, while crowngall may be termed a woody tumor.

This feature of the apricot disease has been found to be constant. It is considered a reliable criterion, by means of which the disease may be readily differentiated from crowngall, with which it is sometimes confused.

To resume the description of the anatomy of the apricot gall: It will be noted from Plates 3 and 4, A, that the wood tissue shows lesions. These consist of irregularly shaped gum pockets, having a dark chestnut color. In young, actively growing galls they have been found to extend from the cambium line into the wood to a distance of 1 to 2 mm. Plate 4, A, representing a young gall on a root, shows the wood riddled by these pockets along the cambium line. This, however, is an extreme case which never occurs in the aerial galls. It seems quite obvious that the disease never works its way deeply into the wood. The fact that the gum in the pockets in the interior of the wood is dry, indicating that the lesions are probably stationary, coupled with the observation that they are often found along the yearly rings of growth, leaves little doubt that these lesions were formed through the extension of lesions from the phloem into the adjacent wood, and have subsequently been separated from the phloem by the interposition of successive layers of wood tissue in the course of the tree's growth.

Scattered through the bast are large numbers of small gum pockets, varying in shape and outline. They range from microscopic dimensions to several millimeters in diameter, often forming confluent lakes, and are packed with gum and disintegrating tissue of a light bay to light chestnut color. Although moist, the contents of these pockets are not watery, but rather firm and will fall out bodily on pressure, leaving empty holes behind. Such gum pockets are very abundant in the bast region immediately below the excrescence, and have been found in lesser quantities throughout the bast at distances of 1 to 2 inches beyond the visible swelling.

The gum becomes more and more abundant towards the exterior in the direction of the cork. The bast is found grading into a region throughly permeated with gum. This region corresponds in its position to that of the normal cork. It is, in fact, continuous with the normal cork beyond

the excrescence, although differing from it histologically. It is in this region that the greatest hypertrophy occurs.

On the exterior of these galls may be found pieces of the original smooth rind of the tree which had been torn loose and pushed outward by the pressure of the proliferating tissue within. The external portion of the tumor tissue soon dies and dries up, becoming hard and brittle. Normally but little gum exudes from the galls, but when they are removed from the tree and soaked in water or glycerin great quantities of gum readily diffuse out.

NORMAL HISTOLOGY OF THE MOORPARK APRICOT

If a cross section is made through a healthy old Moorpark branch and the tissues are followed from the exterior inward, it is found, to begin with, that the epidermis is ruptured and torn owing to the stress of internal growth, and that beneath it there is a relatively wide area of seemingly stratified tissue (Pl. 5, A, B, C,) consisting of layers of flat, tabular or brick-shaped cells. These cells are filled with air, and therefore have a refractive index differing from that of any other tissue. By means of these characters and also by the close arrangement of the cells, allowing no intercellular spaces, this tissue is readily recognizable from any other in the plant system. This is the cork, which, being impervious to water, serves the plant as a protection against excessive evaporation and extreme changes of temperature. Alternating with these cork strata may be found strata of thin-walled cells wider in radial diameter than the cork cells, and angular rather than brick-shaped. This is the cork parenchyma or phelloderm (Pl. 5, D, E, F). Scattered through these tissues, and more abundantly through the cork parenchyma, may be seen clusters of cells whose walls are greatly and uniformly thickened; these are sclerenchyma—or stone cells (Pl. 5, G, H), which serve as a buttressing element to the tissues in which they are found.

These tissues arise from the cork cambium or phellogen, which in the genus *Prunus*, to which the apricot belongs, originates in the subepidermal layer of cells (*8*, *p. 307*).[3] The primary cork cambium does not remain active indefinitely, and a new one is formed from the innermost layer of cells recently laid down by it. This new cork cambium forms cork tissue toward the exterior, and, less frequently, thin-walled parenchyma, or phelloderm tissue, toward the interior. This explains the occasional occurrence of layers of phelloderm between cork strata (Pl. 5). All of these tissues—cork cambium, cork, and cork parenchyma—located between the epidermis and the phloem or bast region underlying them (Pl. 5, I), are collectively spoken of as periderm.

It will be noted that the line of demarcation between the periderm and the phloem is sharp. Rarely, if ever, do we find in the normal apricot plant, cork tissue extending any appreciable distance into the phloem. While small portions of the outermost zone of the primary phloem may occasionally be intercepted by cork layers, the successive strata of cork tissue tend, as a general rule, to preserve their parallel course; the divergent cork strand soon rejoins the tissue from which it arose.

In this feature—namely, the sharp demarcation between phloem and cork tissues—lies the essential difference between the histology of the normal apricot tree and that of the galls.

[3] Reference is made by number (italic) to "Literature cited," p. 59.

Little need be said about the tissues lying beneath the periderm. The phloem, the next internal tissue, is traversed through nearly all its length by the medullary rays, which radiate also through the next tissue, the xylem or wood, and terminate internally in the pith in the center of the tree. The phloem and xylem are separated by the cambium; a single layer of meristematic cells, which, by constant division and subdivision in a radial direction, deposit wood toward the interior and phloem tissue toward the exterior.

PATHOLOGICAL HISTOLOGY

On the outside of the gall remains of the epidermis may be found still attached and, beneath, the periderm with its cork strata alternating occasionally with more or less incomplete strata of phelloderm or cork parenchyma and clusters of sclerenchyma cells, in the same way as has been observed in the normal tissue of the Moorpark apricot. This is the original bark, which, under the diseased condition, has been dislocated from its normal position and pushed to the exterior. When, however, the region where the periderm ends and the phloem begins is reached, it is found that the two kinds of tissue are not sharply marked off as they are in the normal condition, but, instead, strands of cork tissue are found diverging from the normal parallel course and forking off to enter the phloem. Strands of cork tissue have been observed leaving the periderm and penetrating into the phloem region, incidentally crossing the medullary rays and occasionally abutting into the cambium. Plate 6 shows cork tissue deep in the phloem. Note the strands of cork tissue at A and B close to the cambium. Plate 7 shows cork and phloem tissues grown into each other. Note the cork strands at A, B, and C, and the medullary ray D, whose course is obstructed by the cork. Another pathological feature illustrated by this plate is the unusual abundance of sclerenchyma cells in this region (E).

The presence of cork in the midst of phloem tissue—the actual mingling of two kinds of tissue in a more or less complicated manner often resulting in portions of the phloem being surrounded on all sides by cork—constitutes the most striking feature of the pathological histology of the gall tissue. This condition is characteristic of all the tumor growth lying outside of the line which is continuous with the normal bast (Pl. 3, B).

The cork becomes less and less abundant farther inward. The phloem lying immediately outside of the wood, although occasionally invaded by strands of cork tissue, is soon replaced by new phloem formed by the cambium, and the disorganized bast is pushed to the exterior.

The surrounding of phloem tissue on all sides by strata of cork cells several layers deep would in itself be sufficient to result in the death of the inclosed tissue, for cork, being impervious to water, prevents the interchange of nutrients in solution through it. The inclosed tissues would thus be expected to die of starvation.[4] While this may actually be taking place in the tumor, it is generally found that the tissues in the region under consideration are in a much more advanced state of disorganization than can be attributed to the effects of starvation. The

[4] This is what happens normally in the old bark of the oak and elm—for example, where layers of phloem are cut off from water and food supply by deep layers of cork tissue. The isolated phloem tissue soon dies and, together with the cork, becomes furrowed by many clefts and fissures. So it happens in the case of the grapevine and birch, where the dead phloem and surrounding cork slough away after drying.

phloem tissue in the external excrescence is generally found in a rather advanced state of gummous disintegration. While gummous lesions have been observed in this region in various stages, the incipient stages had to be studied from the lesions deeper in the phloem where it is relatively free of cork.

LESIONS IN THE PHLOEM

It will be remembered that the bast lying immediately outside of the wood has many gum pockets scattered through it. Plate 8 shows a cross section through phloem parenchyma with a very small lesion in it (B). This lesion is surrounded by healthy tissue, which is more deeply stained, and beyond the healthy tissue there is another lesion (A). An examination of the lesion B will show it bordered by a layer of flat cells containing many granules. The center of the lesion is occupied by gum (C), while the tissue of this, as well as the other lesion, consists of thin-walled, irregularly shaped cells, apparently undergoing a process of gelatinization.

What happens to the healthy tissue when it becomes diseased may be more clearly understood when Plate 8 is compared with Plate 9, which shows a different plane of the same lesion, illustrating a later stage of the disease. The band of healthy tissue which in Plate 8 is seen separating the lesions at A and B is missing in Plate 9, its place having been taken by the thin-walled gelatinizing cells.

An examination of numerous lesions would seem to indicate that the progress of this phase of the gall is as follows:

The first indication of the disease shown by the affected tissue consists in a thinning of the cell walls.

It would seem that in some cases the surrounding healthy tissue tries to check the advance of the disease by increasing the food content of the cells bordering the lesions, as may be concluded from the denser and more granular state of their protoplasm. This, however, is by no means a constant feature.

The bordering cells, thus richly provided with food, begin, in some cases, to proliferate rapidly. This may be interpreted as an attempt to protect the healthy tissue from the oncoming disease, or at least to delay its advance by throwing into the diseased area, tissue at the rate at which it is consumed. This attempt is never successful.

The cell walls in the interior of the affected tissue continue in the meanwhile to grow thin and stretch, and the contents of the cells continue to gelatinize. Soon the cells collapse, and, with the disorganized protoplasm and cell sap, constitute the "gum" which fills the lesions.

The nuclei seem to be more resistant to the effects of the disease, persisting in the cells at a time when the cytoplasm and cell wall are in an advanced state of gelatinization (Pl. 8 and 9).

No difference has been noted in the way in which the various phloem elements behave under the influence of the disease. When a medullary ray is affected its course is stopped short and its cells undergo the same process of proliferation, gelatinization, and, finally, gummous disintegration, as has been described above.

LESIONS IN THE WOOD

In the gummous lesions in the wood a similar process is taking place. Plate 10, representing a cross section through the xylem, shows a series of confluent lesions extending diagonally across the field. The black

area represents xylem parenchyma and tracheal tubes which disintegrated and became filled with gum. The earlier stages of the disease are illustrated by the tissue bordering the lesion. Note the flat, narrow cells at A, evidently in a state of very rapid multiplication; note, also, the cells about B, which, like the affected cells of the phloem parenchyma, take the stain less deeply than the cells of the surrounding healthy tissue, have thinner cell walls, and are more irregular in shape—apparently undergoing a process of gelatinization similar to that through which the diseased phloem tissue passes in the course of its gummous degeneration. The smaller lesion in the upper left hand corner brings out the same facts. Gummous lesions in the wood have also been found involving wood fibers and pitted ducts. In these cases there was no multiplication of these elements, but merely a gummous breaking down.

There are thus found in this gall of the Moorpark apricot the phenomenon of gummosis on the one hand, and hyperplasia—that is, abnormal and excessive cell division—on the other. No true hypertrophy, in the narrow pathological sense of the term—that is, an abnormal increase in the size of individual cells—has been observed.

PROBABLE COURSE OF THE DISEASE: CONCLUSIONS

In view of the fact that gummous lesions exist in the bast at a distance of from 1 to 2 inches beyond the gall, it seems probable that the first stage of the gall consists in a gummosis of portions of phloem tissue. The outlying region of the phloem just below the periderm is the first to become affected. The gummous lesions may later extend deeper into the phloem and occasionally cross the cambium to enter the wood.

The cork cambium, in the meanwhile, receives a stimulus for abnormal cell division. As a result, cork strands diverge to enter the affected area, in what may be interpreted as an attempt to isolate the lesions or heal them over.

In order to account for the large amount of phloem tissue involved in the tumor, as well as for the general increase in wood in the region underlying it, it must be assumed that the disease is accompanied by a general acceleration of cambial activity. The occasional proliferation of cells bordering the gum pockets is of very minor importance in this connection, and can not possibly be held to account for the large swellings produced.

By replacing the phloem tissue as fast as it is destroyed, or nearly so, the cambium enables the tree to withstand the effects of the disease for long periods of time.

The effect of the disease is to be looked for in a constant loss of indispensable tissue, which must be replaced in order that the tree may escape the danger of being ringed. When the cambium fails to replace the phloem as fast as it is destroyed, the disease is naturally aggravated, for such a condition results in interference with the translocation of manufactured food which must be carried through this tissue.

EXPERIMENTAL DATA

The general features of the histology of the galls were studied from wood-microtome sections. Recently formed tissue from the edges of the galls was sectioned fresh or after soaking in water. Where the material was permeated with gum, as is very often the case, it was soaked

in glycerin. This served to soften the tissue to some extent, and, in general, to render it less brittle, by dissolving part of the gum. To overcome the tendency of many sections to break or crumble before the knife, the exposed surface of the material to be sectioned was covered just before cutting with a thin layer of collodion, which served to hold the loose parts together. This method is recommended by Lee (*4 p. 95*). By these methods a sufficient number of fairly good sections was obtained, ranging in thickness from 15 to 20 μ.

In order to obtain thinner sections for closer and more detailed study, material was embedded in paraffin. Of the several killing and fixing reagents used chromo-acetic acid [5] gave the best results. By this method good sections 7 to 10 μ thick were obtained. The phloem, when free from cork, gave the best results in sectioning. Wood tissue was sectioned with little difficulty by means of the wood-microtome. The following stains were used: Methylene blue, Ziehl's carbol fuchsin, eosin in 95 per cent alcohol, and Delafield's haematoxylin counterstained with the alcoholic eosin. The stain last named was recommended by Durand (*1*) as a differential stain for intercellular mycelium.

ETIOLOGY

ISOLATION AND INOCULATION EXPERIMENTS

In order to ascertain what the causal agent of this disease might be, a fairly extensive series of culture experiments was carried out. Fresh galls were obtained on several occasions between January 24 and April 7, 1916, from San Jose and Hayward, Calif. The rough surfaces of the galls were cut off; they were then washed in running water and placed in 95 per cent alcohol for five minutes, and finally soaked in an aqueous solution of corrosive sublimate, 1:1000, for from 20 to 30 minutes. The corrosive sublimate was then rinsed off with sterile water, and bits of tissue were removed from the interior of the galls and planted in various media, care being taken to observe all aseptic precautions. Tissue bordering the gum pockets in the bast where the latter is free from cork was generally used. It was soon found absolutely necessary to avoid, as far as possible, the carrying of gum along with the sections used in planting, on account of the inhibitive effect which the gum seemed to have on the growth of microorganisms. The plantings were made in standard nutrient agar, standard nutrient broth, and sterile water. Bacterial dilution plates were also made in standard nutrient agar.

The bacterial dilution plates and the plantings in standard nutrient broth and agar failed to give any positive results. Occasionally fungous mycelium developed on some of the sections in standard nutrient agar, but was either swamped by gum oozing out from them or failed to grow when transferred to other media.

What is believed to be the causal agent of this disease was obtained by planting a number of sections in tubes of sterile water. These were left overnight. The following morning, when the water was found to have changed to a rich brown color, it was thought desirable to transfer the sections to a medium free from any pathological products. They were accordingly placed in flasks containing some 30 cc. of standard nutrient broth. In one of these flasks a thrifty fungous colony developed,

[5] Chromic acid, 0.05 per cent; glacial acetic acid (99 per cent) 0.1 per cent.

which was soon isolated in pure culture; in three weeks' time it formed spores. It was identified as belonging to the genus Monochaetia Sacc. Inoculations were made with pure cultures into 2-year-old nursery trees of several varieties of apricot and cherry. These plants were growing in pots, and, with the exception of two which were placed in the greenhouse, were standing in the open. In all the inoculation experiments the surface of the region to be inoculated was carefully disinfected with corrosive sublimate (1:1000) for from 10 to 20 minutes, and the disinfectant was then washed off with sterile water. A slanting cut was made with a flamed knife, and pieces of fungous mycelium with spores were inserted. About one control, consisting of a cut which was not inoculated, was allowed for every three inoculations. The treated area was then wrapped with waxed paper as a precaution against external infection and drying out. The results of the inoculations may be seen from the following table:

Trees inoculated.	Date of inoculation.	Number of inoculations.	Number of controls.	Results of inoculations.	Controls.
Moorpark apricot..	Mar. 16 to 18...	9	3	8 positive; 1 doubtful.	Healed over.
Do.[a].....	.....do.....	9	3	0 positive....	Do.
Royal apricot.....	Feb. 16 to 26....	4	1	4 positive....	Do.
Early Newcastle apricot.	.....do.....	3	1	3 positive....	Do.
Royal Ann cherry.	Feb. 14 to 16....	4	2	.....do.....	Do.

[a] This tree was in a very poor condition, which may account for the negative result obtained from the inoculations.

The positive results consisted in the formation in the course of two or three weeks of small, heavily gumming cankers, which became stationary at the end of several months and healed over the following year. No gall growth was formed on any of the inoculated young trees.[6]

On February 25, 1916, 12 inoculations were made in four branches of an old apricot tree of an unidentified variety, probably a seedling. Branches about 4 to 6 years old were chosen for the purpose. In this case the inoculations were made, not into slanting cuts, but into notches formed by making two deep parallel cuts across the branch and removing the intervening bit of bark and wood tissue. This later afforded a good opportunity for comparing the normal callousing of the wounds in the controls with the gall growths which formed in the inoculations and for differentiating them. In the course of about three weeks small cankers formed about all the inoculations, accompanied by more or less abundant gumming. The lesions continued in a state of constant gumming for nearly six weeks without showing any other changes. About the first week in April, however, when the growing season had well started, a swelling became noticeable around the gumming lesions, accompanied by a longitudinal cracking of the bark. It soon became evident that new tissue was being formed there. The throwing out of

[6] Similar results were obtained by Miss E. H. Phillips on Royal apricot from inoculations made in March, 1916.

tissue from the lips of the gumming wounds continued until the first week in June, after which no change took place. Of a total of 12 inoculations 9 resulted in eruptions or small galls, 3 formed small gumming cankers which remained stationary throughout, while the controls healed over normally. Unfortunately, the tree bearing the inoculations was pulled up in connection with the building of a new hall for the College of Agriculture. Plate 11 shows the condition of the 9 positive inoculations on August 10, when they were photographed.

An examination of the callous which had formed on the control cuts showed it to consist largely of wood tissue which had developed to heal the cut. In the lesions produced by the inoculations, however, no such wood tissue was formed. The interior was filled with gum and the swelling was found to be due to growth in the bark. The inoculations with *Monochaetia* sp. into old apricot tissue have thus resulted in abnormal growth identical in all its essential features with that of the galls on old Moorpark apricot trees.

It is interesting to note in this connection that the same organism which produced gumming cankers, when inoculated into young plants without any formation of abnormal growth, formed when inoculated into the branches of an old tree gumming lesions at first, which were later followed by abnormal growth. This would seem to indicate that the first stage of the disease consists in the breaking down of the affected tissue, resulting in a gummosis, and that this period is followed by one in which the plant attempts to outgrow the lesion. The formation of galls results. Another fact shown by the inoculation experiment is that galls may be artificially produced on varieties other than the Moorpark, to which the disease is practically restricted in nature.

On May 19, 1916, when it became apparent that the inoculations on the old apricot tree promised to produce galls, a larger number of inoculations, amounting to a total of 100, were made on old apricot trees of the Moorpark and Blenheim varieties, at Hayward, Calif. Several of these inoculations were believed at the time to have been successful, for they were gumming somewhat more abundantly than were most of the controls. None, however, produced galls. By the end of the summer all of the inoculations showed signs of healing over. The negative result of this experiment is attributed to the dry and consequently unfavorable weather conditions existing at the time the inoculations were made, and afterward. As has already been pointed out, infection in nature takes place during the winter and early spring, and the spread of the disease is proportional, generally speaking, to the humidity of the season.

REISOLATION OF MONOCHAETIA SP.

In the writer's absence the work of reisolating the causal agent from the eruptions resulting from the inoculations with *Monochaetia* sp. was performed by Miss Helen Czarnecki.

An inoculated branch was soaked for three minutes in alcoholic corrosive sublimate (1:2000 $HgCl_2$, in 50 per cent alcohol). The rough surface was cut off with a flamed knife and bits of tissue were removed from the interior of the bark and planted in standard nutrient broth. By this method pure cultures of *Monochaetia* sp. were obtained in five cases out of seven.

Another inoculated branch was soaked in 95 per cent alcohol for five minutes, then passed through the flame and placed in a moist chamber.

A delicate whitish mycelium was noted four days afterwards forming on the inoculated cuts. In the course of two weeks acervuli, bearing spores typical of *Monochaeti* sp., formed on the lips of the three eruptions. It was from this source, as well as from the acervuli which were found on the margins of the eruptions on one of the inoculated branches which was left untreated, that spores were obtained for the study of the morphology of the fungus.

Spores of *Monochaetia* sp. were recognized in one or two instances among a variety of spores of different fungi obtained from the surface of the galls on Moorpark apricots.

A microscopic search was made without success for organisms in the tissues of the gall. This negative result may be attributed in large measure to the extreme difficulty of obtaining sections thin enough and sufficiently well preserved for study, particularly from the region where cork and phloem are mixed. The phloem when free of cork is much more easily sectioned, and in this region a careful search was made for mycelium, without any positive result. In this connection the combined Delafield's haematoxylin and alcohol-eosin stain, recommended by Durand (*1*) for the purpose of bringing out intercellular mycelium, was generally used. Further experimentation with a greater variety of stains, and perhaps the celloidin method of imbedding, would be necessary to bring this phase of the work to a successful completion.

While the chain of evidence required by Koch's rules for etiology and proof is incomplete, the writer believes that *Monochaetia* sp. is the causal agent of the Moorpark apricot gall. This belief is based on the successful inoculation and reisolation experiments discussed previously.

THEORY CONCERNING THE MODE OF INFECTION

No experimental data are at hand on the manner in which the parasite[1] finds entrance into the tissues of the host. It may be worth while, none the less, to record some observations made on this subject.

In no case have any eruptions been found in connection with wounds of mechanical or animal origin. The young galls are always associated with cracks in the bark of the tree. While these cracks may be the effect of the extrusion of gall tissue, and may not represent the road through which the parasite makes its way into the bast, a curious correlation has been noted between the extent of the cracking which takes place in the bark of the Moorpark apricot and the frequency of the disease in this variety. By comparison with the relatively resistant Blenheim variety the bark of the Moorpark shows not only a more extensive but also a deeper cracking. Although many Blenheim trees have been found which could not be distinguished from Moorparks on the basis of the appearance of the bark, the difference generally holds good and seems quite obvious when the comparison is made between groups of trees, as, for instance, between adjacent rows of the two varieties. No less suggestive is the fact that the disease attacks the trees during old age, a period in which cracking of the bark is characteristic, and never occurs on young trees. Thus, while the question of the mode of infection must be left in doubt until settled by empirical evidence, the theory that the parasite enters the tissues of the host through cracks which form

[1] The writer's experience with this disease leaves him in no doubt that it is of parasitic origin. Support of this view is also given by results obtained from crude inoculations made by Earl Morris in 1917, at San Jose, Calif., who reproduced the galls by inserting bits of gall tissue into the bark of healthy apricot trees.

naturally in the bark and which expose the deeper layers of the phloem to infection, gives a plausible explanation of the susceptibility of the Moorpark variety, and the occurrence of the disease exclusively in old trees.

CONTROL WORK

The treatment which naturally suggested itself in this connection was that of removing the galls by excision. Fifty galls of various sizes were cut off on May 19, 1916. Among these were very small eruptions just making their appearance, as well as older galls up to 8 inches in length. The bark surrounding the visible swellings for a distance of 1 to 2 inches was carefully examined. In most cases it was found to contain gum pockets and was therefore removed. It was thought necessary to remove some of the tissue which showed no visible signs of disease, along with the diseased tissue. All wood with fresh gum pockets in it was gouged out. Relatively wide areas filled with dry gum were often found extending into the wood. While these lesions were not considered a possible source of reinfection, they were thoroughly gouged out as a provision against wood decay. The wounds formed in this manner were smeared with Bordeaux paste (bluestone, 1 pound to 1 gallon of water; quicklime, 2 pounds to ½ gallon of water).

None of these galls returned during the years 1916 and 1917. It is safe to say, therefore, that the disease can be held in check in the manner described in the foregoing paragraph. This can be accomplished at a relatively low expense if the galls are removed while small. It is hardly necessary to point out in this connection that limbs which have been encircled by the gall, or nearly so, are past treatment. A complete removal of affected tissue in this case would necessitate the ringing of the limb, which would result in its death.

THE PROBABLE CAUSAL AGENT

Monochaetia rosenwaldia Khaz, n. sp.[8]

A careful comparison of the characteristics of this species of *Monochaetia* with those of all the species of the genus listed by Saccardo (7), as well as with a number of other newly described and yet unlisted species, seems to show that the parasite in question is a new species. Not only is there no other species of this genus resembling it in its mode of parasitism, but none of them is identical with it in morphological characters.

The writer takes occasion, therefore, to name this fungus *Monochaetia rosenwaldia* Khaz. after Julius Rosenwald of Chicago, Ill., to whom he

[8] *Monochaetia rosenwaldia* belongs to the Imperfect Fungi. The genus is characterized by acervuli and conidia which are at least two-septate, the apical and basal cells of which are more or less hyaline. The most distinctive feature of this genus, however, which separates it from its ally, the genus Pestalozzia De Not, lies in the matter of apical appendages, of which Monochaetia has only one, while the conidia of Pestalozzia have several cilia. It was on the basis of this purely abitrary distinction that Saccardo raised the group from a subgenus of Pestalozzia to the generic rank (*7, p. 485; 5, p. 411*).

The genus Monochaetia is composed largely of saprophytic species. The genus Pestalozzia, on the other hand, contains a number of plant parasites, and of these there are two whose mode of parasitism seems to be more or less similar to that of *Monochaetia rosenwaldia*. They are:

(1) *Pestalozzia hartigii*, generally considered the cause of a disease affecting a variety of tree and shrub seedlings in the nursery which manifests itself in the constriction of the stem just above the soil and results in the death of the affected plant. Fischer (*2*) reports that he failed to obtain infection from inoculation with this fungus into an extended range of seedlings.

(2) *Pestalozzia tumefaciens* P. Henn., to which Hennings (*3*) ascribes a disease found by him on several species of Abies. This disease manifests itself by the formation of "gall-like swellings" on the branches. The internal consistency of these galls is also described, but, unfortunately, in rather indefinite, nontechnical terms. Since no anatomical features are mentioned in his description, no conclusion can be drawn with regard to the similarity of that disease to the apricot gall.

desires to show gratitude, and, more especially, appreciation of the interest shown by this philanthropist in the development of agricultural science in Palestine, at a time when such interest was generally lacking.

MORPHOLOGICAL CHARACTERS

ACERVULI.—Black, irregular in shape, frequently circular, sometimes confluent, often submerged in solid artificial media, erumpent. Of all sizes, from tiny specks to 1, 1½, and 2 mm., or larger masses when confluent.

CONIDIA [9].—Fusoid or oblong-fusoid, straight or curved, or ovoid; three-septate, rarely four- or five-septate. Constricted at the septa. The color of mature spores, umber, of a shade lighter than Saccardo's (7). Young spores grade from hyaline or subhyaline to dark olive-buff.[10] Basal cell hyaline, occasionally subhyaline; terminal, hyaline.

APPENDAGES.—Mostly single and curved, occasionally straight and oblique, hyaline. Forked appendages not exceeding 6 per cent. Extremes for length for 89 appendages were 3 to 36 μ; 8 μ was the standard length. The appendages range in width from 1 to 2 μ at the base, tapering.

SPORE DIMENSIONS.—In 120 spores the extreme measurements for length and width were, respectively, 12 to 27 μ and 5 to 10 μ; the standard, or the most common, values for length and width were 16 to 17.5 μ and 6.5 to 7 μ, respectively.[11]

Plate 12 shows some characteristic spores of *Monochaetia rosenwaldia*. Of these, the spores lettered A, C, E, and J represent the most common types. Plate 12, K–P, shows irregular forms, varying from the standard with regard to the number of the cells. Such variations were very rare. Q and W, on the same plate, show variations in the apical appendages.

CULTURAL CHARACTERS

ON STANDARD NUTRIENT AGAR.—Plate 13, A, shows a 6-day-old colony in a Petri dish culture of this medium at a magnification of ×2. The growth of the fungus, which is very vigorous at first, often becomes arrested. Acervuli form rarely. The mycelium penetrates but little into the interior of this medium.

ON PRUNE JUICE AGAR.—Plate 13, B, shows a 9-day-old colony in a Petri dish culture of this medium at a magnification of approximately ×1⅓. Note the zonate character of the growth. This is a feature which has not been observed on the other media. Note also the formation of acervuli from white masses of mycelium. The acervuli on this medium are often globular and raised.

ON SHEAR'S AGAR.—The growth of the fungus takes place largely in the interior of the medium; only occasionally does the mycelium spread on the surface. The acervuli are erumpent or submerged, forming at about the same time as in the previous medium—namely, when the colony is about 10 days old. A faint zonation is sometimes noticeable in very young cultures but soon disappears.

GROWTH IN STANDARD NUTRIENT BROTH.—At first somewhat zonate. When the surface of the liquid has been reached a white, woolly pellicle is formed. Acervuli at first usually submerged in the mycelium, erumpent at maturity. They rarely form before three to four weeks.

GROWTH ON INFUSIONS OF MOORPARK APRICOT LEAVES.—Very vigorous and rapid.

A series of cultures was carried on in infusions of beets, carrots, turnips, and Irish potatoes. No distinction could be noticed in the growth produced in these media. Apart from the acervuli, which in these cultures formed somewhat earlier than on the other media, the growth was not characteristic. Acervuli were recognizable at the end of the week and formed in large abundance, notably on Irish potatoes. In this medium nearly all the vegetative mycelium was soon covered with continuous jet-black masses of spores. These vegetable media may be used to advantage when large quantities of spores are required.

[9] The material for the study of the spores was obtained from acervuli on the inoculations.

[10] RIDGWAY, ROBERT. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

[11] The suggestion for the use of this mode of expression in the description of spores was first made by Dr. E. P. Meinecke (6) in preference to the usual way, in which only the extreme dimensions are stated. It is obvious that a much better idea of the size of the objects can be formed when the standard or most common dimensions are taken into consideration along with the extremes.

GERMINATION STUDIES

Plate 12, Z, A′, B′, shows the method of germination of *Monochaetia rosenwaldia*. Note that the contents of the spore swell up to such an extent as to rupture the cell wall. This is followed by the extrusion of a very robust, frequently rotund, sometimes double "germ tube." Germination took place in most cases after 24 hours, and was followed by vigorous growth. No conidia formed in the hanging drop cultures; instead, a peculiar chlamydospore formation was noted at about the time when the mycelium ceased to grow—that is, some three to four weeks after germination had started (Pl. 12, C′).

Since no conidia formed in the hanging-drop cultures, it was necessary to study the method of spore formation from material obtained from acervuli in other cultures. This method would seem to be as follows:

Tips of some hyphae become rounded and swollen. These globular inflations soon become elongate, and are in the meanwhile separated from the sporophore by a septum. Gradually the other three septa form across the spore, while the two central cells pass through the grades of color already mentioned. The appendage at the apex seems to form last of all, by the attenuation of the apical cell or the prolongation of its walls. Plate 12 shows some mature spores on their sporophores in X, while Z illustrates some of the earlier stages in the formation of the conidia.

In view of the fact that some investigators make the length of the sporophores a basis for specific distinction, measurements were taken of a number of these for comparison. Material grown in artificial media had to be used in this connection, for the sporophores in the acervuli on the inoculated branches, which supplied most of the material for the study of the morphology of the fungus, had shed their spores, and could, therefore, no more be distinguished from ordinary hyphae. Measurements of the sporophores are as follows:

Material grown on an infusion of Moorpark apricot leaves: Extremes for length for 30 sporophores were 7 to 62.5 μ; 23.5 μ was the most common measurement for length. Material grown on an infusion of Irish potatoes: Extremes of length for 20 sporophores were 8 to 26 μ; 13.5, 19.5, and 26 μ were equally common measurements.

The sporophores varied greatly in width in the same medium. They were often narrowest about midway and frequently tapering toward the base of the conidium; 1.5 to 4 μ seemed to be the range for the medium widths of most of the sporophores.

These measurements indicate clearly the great variability of the sporophores in different media as well as in the same medium, a fact which is by no means surprising if it is remembered that the sporophore is after all a purely vegetative portion of fungus mycelium. The value of such measurements for the purpose of specific distinction seems therefore doubtful.

SUMMARY

(1) The apricot gall disease, which is more striking than injurious, is readily distinguished from "aerial crowngall," with which it is sometimes confused, through the fact that the galls are bark out-growths in which the wood tissue is not mingled; whereas in crowngall the wood is involved in the formation of the tumor. Unlike the tumors in crowngall, the galls in this disease are thoroughly permeated with gum.

(2) The most characteristic feature of the histology of this disease, apart from the phenomena of gummosis and hyperplasia, is the divergence of cork strands from their normal course on the exterior of the phloem to penetrate the phloem tissue to relatively great distances, reaching sometimes close to the cambium.

(3) The etiology of the disease has not been established with absolute certainty. A fungus, a species of the genus Monochaetia Sacc., was isolated in pure culture from the interior of the galls. When inoculated into the limbs of a healthy old apricot tree, galls were produced which were identical in their gross anatomy with the natural galls. From these the fungus was readily reisolated in pure culture, and reinoculation experiments started. Unfortunately, the trees on which these were made were pulled up before the galls had time to form. The tree on which the original inoculations were made was also pulled up before the resulting galls had become very large. A microscopic search for organisms in the tissues of the galls failed to reveal the presence of either fungous mycelium or bacteria.

(4) No young trees were found affected in nature. Inoculations into young trees resulted in gumming cankers which after several months became stationary, but no galls were formed.

(5) Control measures based on excision and the application of Bordeaux paste gave fully satisfactory results.

(6) The results of a search of the available literature, both with regard to the diseases described on the apricot and to those attributed to members of the genus Monochaetia and its close ally Pestalozzia, seem to justify the belief that this disease has never been described before.

(7) Similarly, *Monochaetia rosenwaldia*, which is believed to be the causal agent of the disease, seems different morphologically from the known species of the genus whose descriptions are available. In view of this fact and particularly in view of the parasitism of this organism, the like of which is unknown among members of the genus Monochaetia, it is regarded as a new species.

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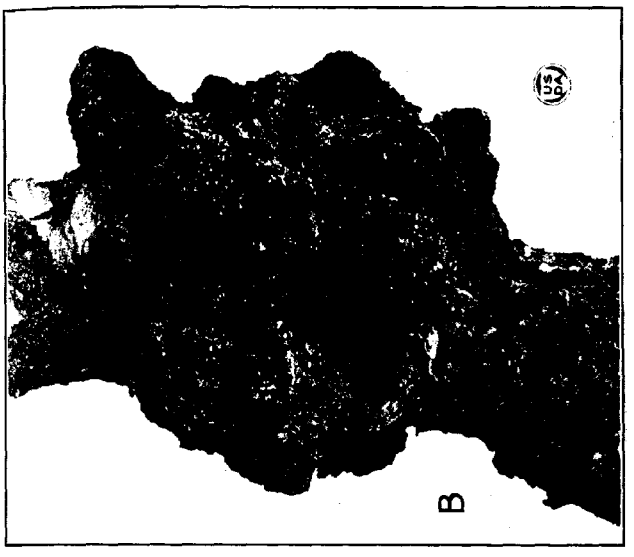
PLATE 1

A.—Young galls on a limb of a Moorpark apricot tree. ×⅜.
B.—A limb of a Moorpark apricot tree encircled by a gall.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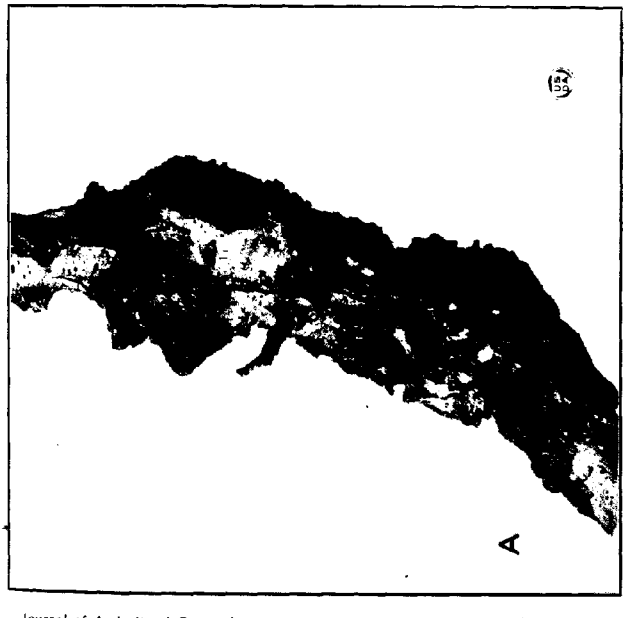
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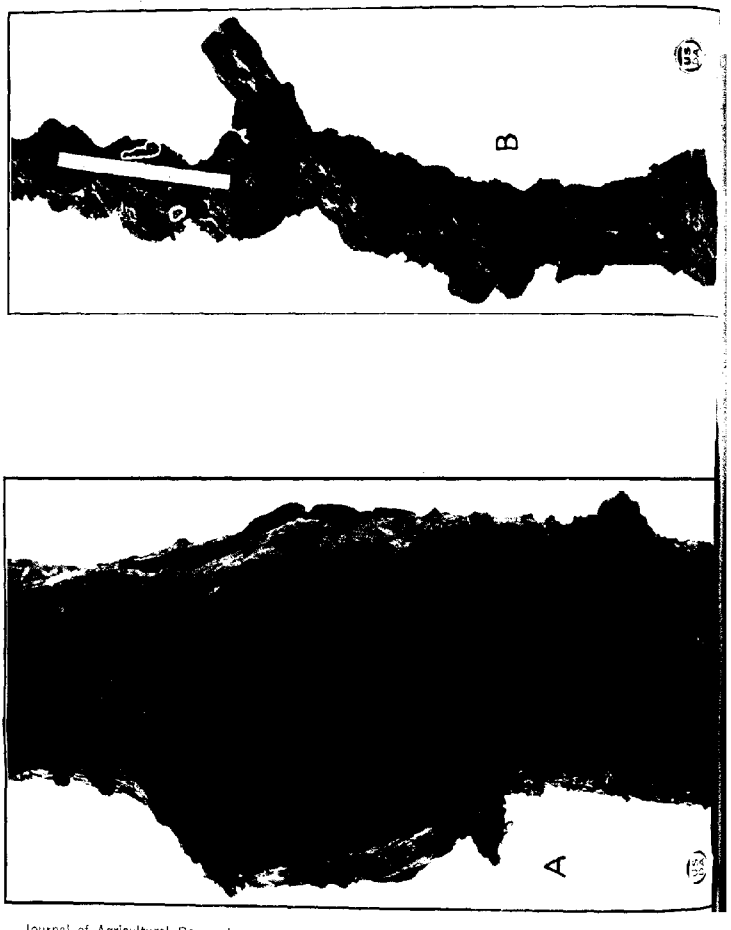
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PLATE 2

A.—An affected root of a Moorpark apricot tree. Somewhat reduced.
B.—A badly affected limb of a Moorpark apricot tree.

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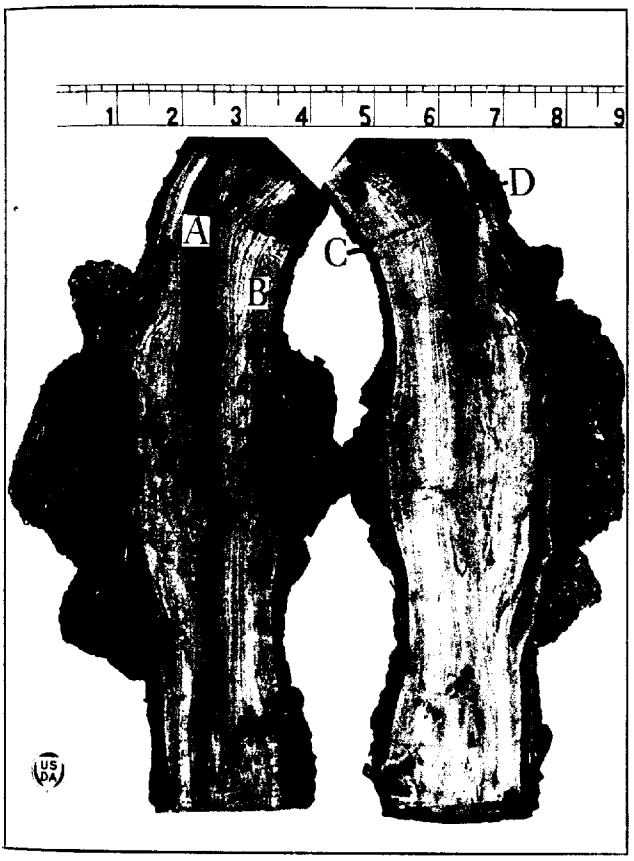
PLATE 3

Longitudinal section of the affected limb shown in Plate 1, B.
A.—Wood.
B.—Bast.
C.—Cambium line.
D.—Cork.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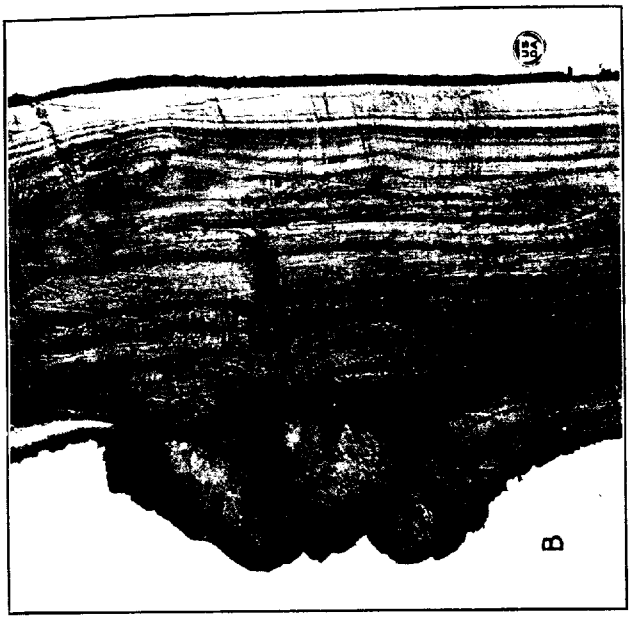


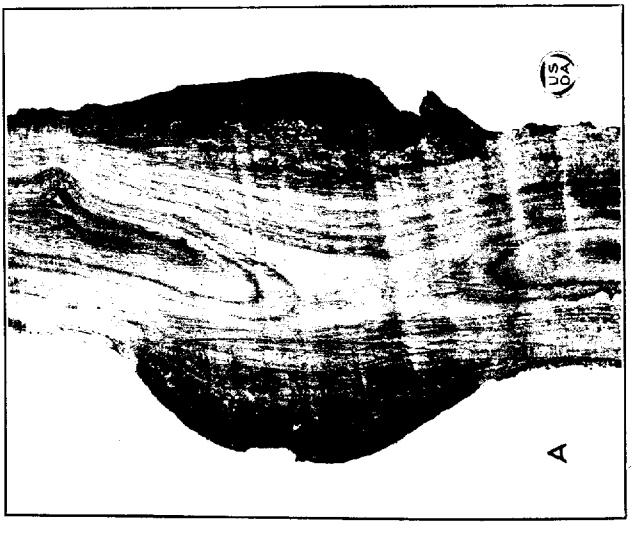


PLATE 4

A.—Longitudinal section of affected root shown in Plate 2, A.
B.—Longitudinal section through a limb bearing a gall caused by *Bacterium tumefaciens*.

PLATE 5

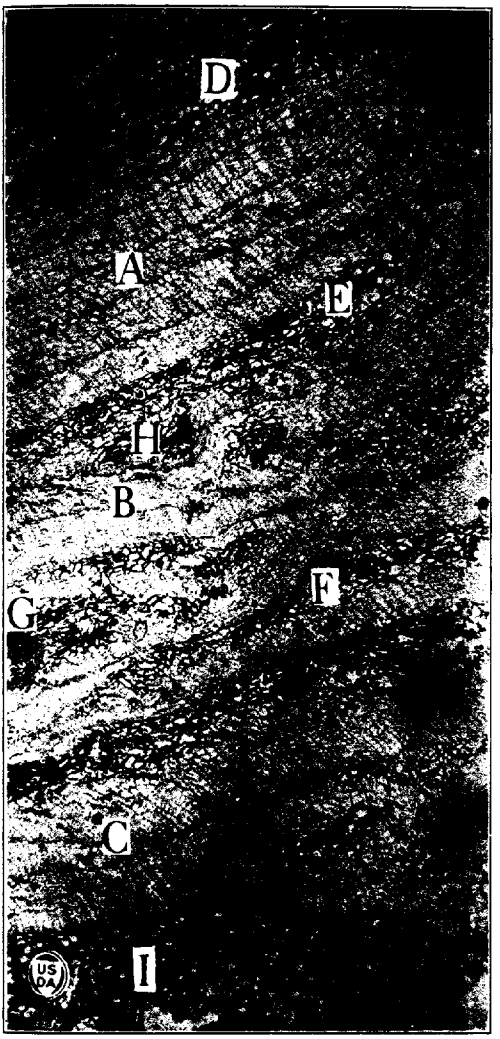
Cross section through the normal bark of a limb of a Moorpark apricot tree. Only an intermediate area is shown, the innermost and outermost portions of the bark having been left out. (Paraffin section 10 μ thick, stained with Delafield's haematoxylin.)

A, B, C.—Strata of cork tissue.

D, E, F.—Cork parenchyma, or phelloderm.

G, H.—Sclerenchyma or stone cells.

I.—Phloem or bast.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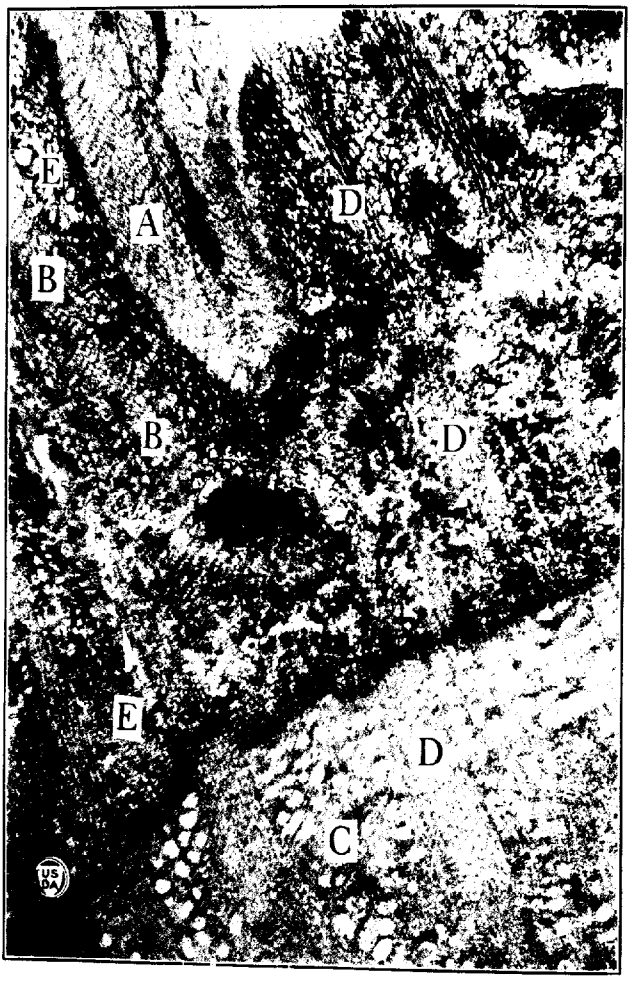
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PLATE 6

View of cross section through gall tissue. (Unstained wood-microtome section 15 to 20 μ thick.)

A, B.—Strands of cork tissue.
C.—Xylem or wood.
D.—Medullary rays.
E.—Phloem or bast.

PLATE 7

View of another cross section through gall tissue. (Paraffins section 10 μ thick, stained with Ziehl's carbol fuchsin.)

A, B, C.—Strands of cork tissue.

D.—Medullary ray.

E.—Sclerenchyma.


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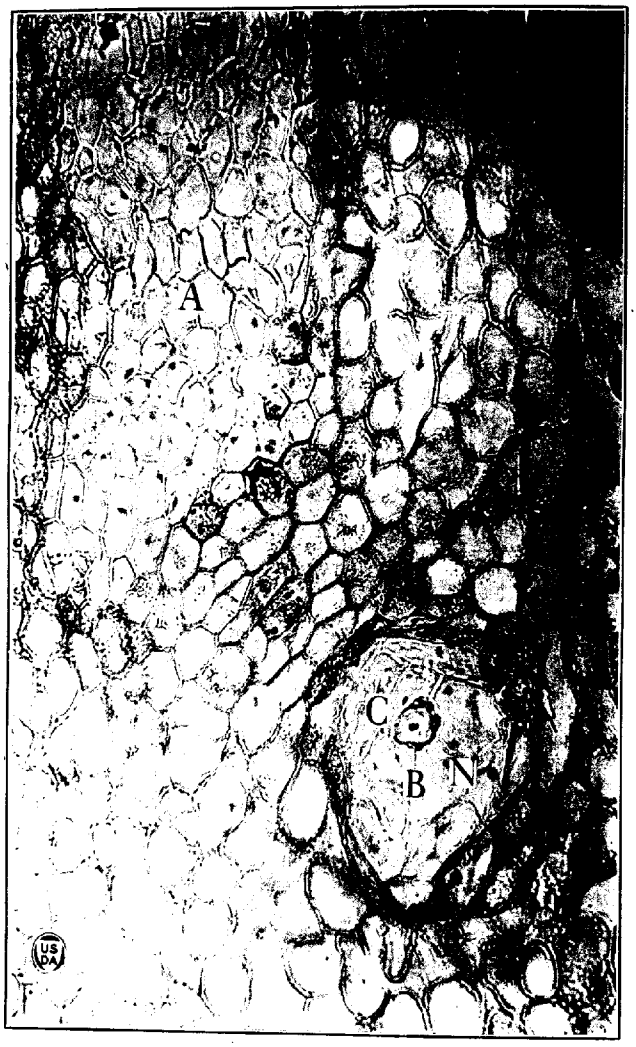
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PLATE 8

Cross section through diseased phloem-parenchyma. (Paraffin section 7 μ thick, stained with Delafield's haematoxylin and counterstained with alcohol eosin.)

A, B.—Lesions.

C.—Gum.

N.—Nucleus.

PLATE 9

More advanced stage of the disease shown in Plate 8.

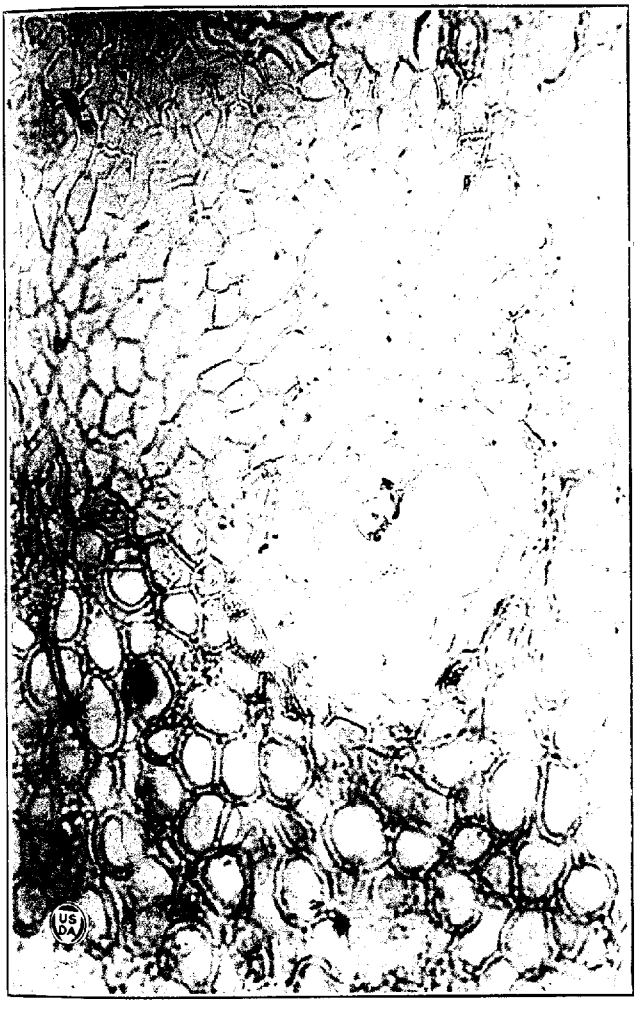


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PLATE 10

Cross section through diseased wood. (Wood-microtome section 10 μ thick, stained with Ziehl's carbol fuchsin.) The black areas denote lesions.

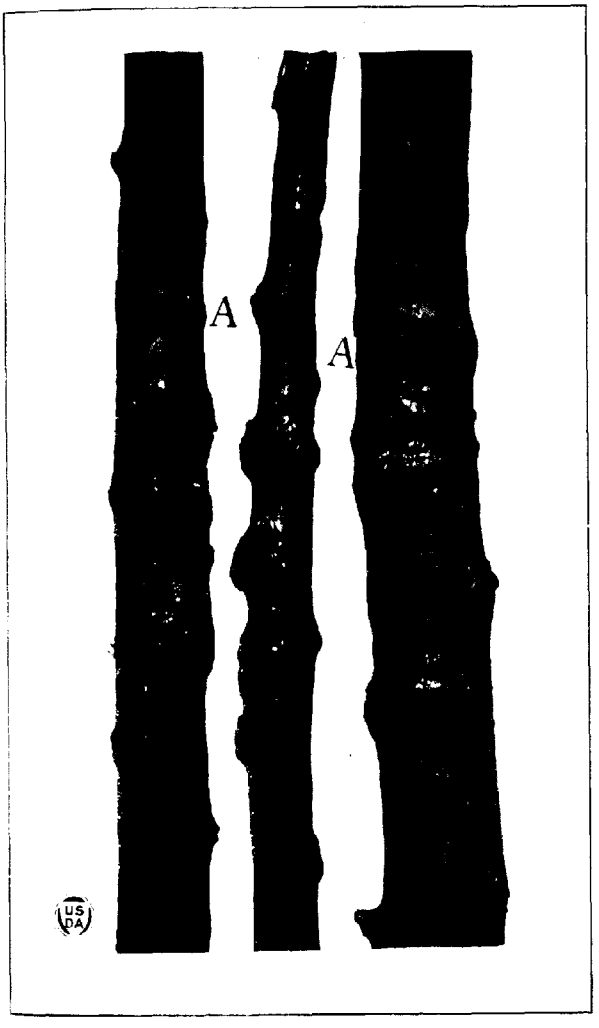
A.—Rapidly proliferating cells.

B.—Gelatinizing tissue.

C.—Affected tracheae.

PLATE II

Incipient galls resulting from the inoculation of pure cultures of *Monochaetia* sp. into the branches of an old apricot tree Slightly reduced. A, controls. (Photographed by Miss Helen Czarnecki.)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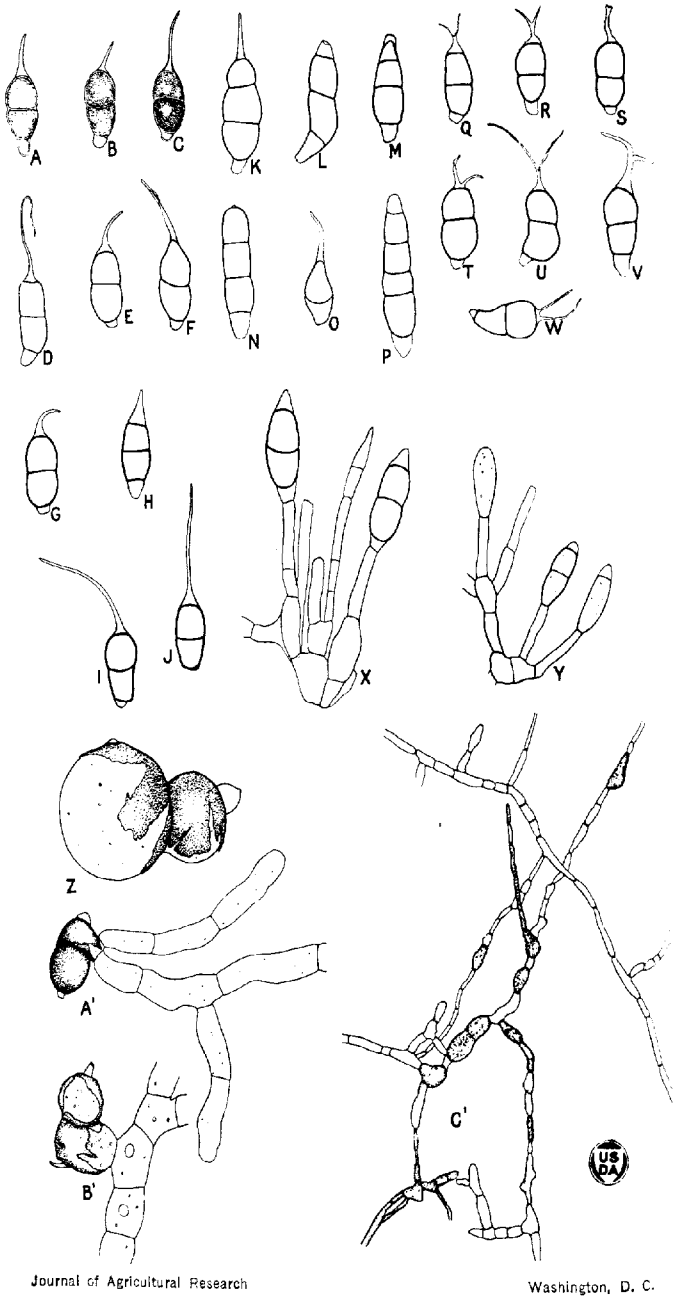
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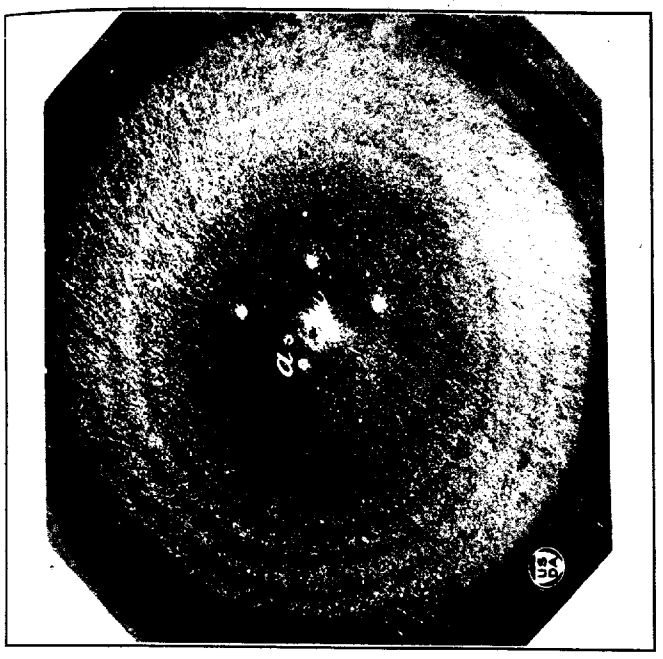
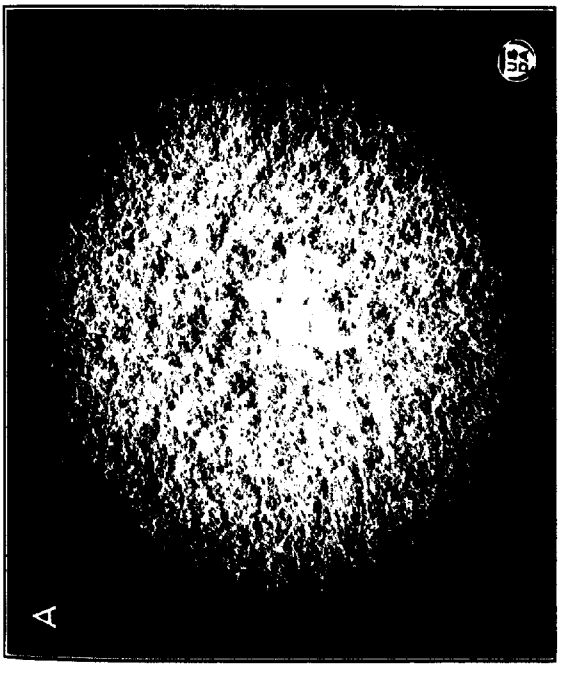
PLATE 12

A–J.—Characteristic spores of *Monochaetia* sp., from acervuli of the inoculations of February 25, 1916.
K–P.—Departures from the standard type with regard to number of cells.
Q–W.—Variant types in the matter of apical appendages.
X, Y.—Conidiophores with young and old condia.
Z, A′, B′.—Germinations of condia.
C′.—Chlamydospore formation in an old hanging drop culture.

PLATE 13

A.—Six-day-old colony of *Monochaetia* sp. on standard nutrient agar. × 2.
B.—Nine-day-old colony of *Monochaetia* sp. on prune juice agar *a*, acervuli. Note zonation. Approximately × 1⅜.

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NOTES ON THE BIOLOGY OF THE CADELLE, TENEBROIDES MAURITANICUS LINNÉ[1]

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INTRODUCTION

The cadelle, *Tenebroides mauritanicus* Linné, is one of the commoner insect pests in stored grain and grain products. Although well known as a grain pest since the latter part of the eighteenth century, but little careful study has been made of its life history and habits and much uncertainty exists regarding them. It is with the object of clearing up some of these uncertainties that the following brief notes are presented.

OVERWINTERING AND EMERGENCE IN SPRING

In the vicinity of the District of Columbia the cadelle overwinters in the adult and larval stages. Mature larvæ that overwinter transform in the spring and large numbers of the newly emerged adults have been observed in May and June. These adults soon mate and shortly thereafter oviposition begins. Freshly emerged adults collected May 25, 1922, began laying eggs July 1, 1922. This would seem to indicate a rather long preoviposition period, at least in the spring; however, female beetles that emerged later in the summer began laying eggs two weeks after emergence. Beetles that overwinter begin laying eggs with the first warm weather of spring.

OVIPOSITION

The beetles either lay their eggs loosely in the flour or other food material or tuck them into a crevice of some sort. They prefer to place them in some protected situation if possible, and advantage was taken of this habit to observe the number of eggs laid. Small pieces of sheet cork were fastened together with paper clips and placed on top of the food in the jar in which the female beetle was kept. The beetle invariably placed her eggs in batches between the sheets of cork, and it was a simple matter to change the cork each day and count the eggs laid.

The eggs are placed side by side in batches containing usually from 10 to 40 eggs. Table I gives a few records of oviposition.

[1] Accepted for publication Aug. 3, 1923.

TABLE I.—*Oviposition records of Tenebroides mauritanicus*

Date.	Mean temperature.	Number of eggs laid by Beetle No.—											
		330[a f]	331[a g]	332[a h]	336[a i]	337[a j]	339[a h]	340[a h]	542[b h]	543[c h]	609[d h]	658[e h]	659[d h]
1922.	°F.												
July 1	85	30	13	....	23	14	....	12	....	....	....	....	....
2	86	....	.....	35	....	32	30	....	....	....	....	....	....
3	84	50	40	32	40	....	....	23	....	....	....	....	....
4	87	30	.....	50	....	18	17	17	....	....	....	....	....
6	73	32	24	....	21	13	13	14	....	....	....	....	....
8	79	18	.....	23	....	....	....	....	....	....	....	....	....
9	80	22	.....	18	....	18	....	....	....	....	....	....	....
10	79	7	.....	....	15	....	....	....	....	....	....	....	....
11	82	11	8	15	6	....	....	23	....	....	....	....	....
13	85	....	40	....	....	....	13	....	....	....	....	....	....
14	75	6	.....	....	....	20	....	34	....	....	....	....	....
15	73	....	25	....	....	....	....	16	....	....	....	....	....
16	77	27	20	....	....	12	....	36	....	....	....	....	....
17	79	16	32	....	....	....	....	....	....	....	....	....	....
18	80	....	40	....	....	34	....	....	....	....	....	....	....
19	80	....	....	....	....	....	39	....	....	....	....	....	....
20	78	31	27	....	36	....	....	....	....	....	....	....	....
21	76	....	10	....	....	....	....	....	....	....	....	....	....
22	79	11	.....	....	....	....	23	....	....	....	....	....	....
23	82	35	14	....	29	....	....	....	....	....	....	....	....
24	86	....	.....	....	....	....	18	12	....	....	....	....	....
25	80	....	32	....	....	....	....	....	....	....	....	....	....
26	75	....	.....	....	....	....	23	....	....	....	....	....	....
27	77	22	24	10	....	24	....	....	....	....	....	....	....
28	81	....	29	....	....	....	....	....	....	....	....	....	....
29	79	17	.....	....	....	....	....	....	....	....	....	....	....
30	79	12	27	....	....	48	....	....	....	....	....	....	....
31	77	....	46	30	30	....	....	....	....	....	....	....	....
Aug. 1	78	....	16	....	12	....	....	6	....	....	....	....	....
2	77	9	20	....	19	....	....	....	....	....	....	....	....
3	78	26	.....	15	....	....	....	....	....	....	....	....	....
4	77	....	21	....	....	....	....	....	....	....	....	....	....
6	80	....	12	....	....	12	....	....	....	....	....	....	....
7	80	....	19	....	23	26	....	....	....	....	....	....	....
8	78	....	22	6	....	....	....	....	40	....	....	....	....
9	72	17	.....	12	....	....	....	....	16	....	....	....	....
10	73	....	.....	....	27	....	....	....	....	....	....	....	....
11	72	....	.....	13	....	23	....	....	....	12	....	....	....
12	70	7	.....	....	....	....	....	....	....	....	....	....	....
13	70	....	12	....	....	23	....	....	....	13	....	....	....
14	75	21	8	....	....	24	....	....	25	....	....	....	....
16	80	....	23	....	13	....	....	35	20	....	....	....	....
17	79	....	12	....	....	....	....	16	....	....	....	....	....
18	83	....	....	....	27	44	....	....	10	40	....	....	....
19	81	....	36	....	....	....	....	20	14	....	....	....	....
20	75	....	34	10	6	....	....	....	26	....	....	....	....
21	71	....	48	20	....	....	....	40	5	....	....	....	....
22	72	24	.....	....	....	....	....	....	12	8	7	....	....
23	75	11	18	....	....	....	....	....	4	....	10	....	....

[f] Adult emerged before May 25, 1922; adult died Sept. 24, 1922.
[a g] Adult emerged before May 25, 1922; adult died Dec. 2, 1922.
[a h] Adult emerged before May 25, 1922; adult still living.
[a i] Adult emerged before May 25, 1922; adult escaped Oct. 5, 1922.
[a j] Adult emerged before May 25, 1922; adult died Nov. 28, 1922.
[b h] Adult emerged July 25, 1922; adult still living.
[c h] Adult emerged July 28, 1922; adult still living.
[d h] Adult emerged Aug. 7, 1922; adult still living.
[e h] Adult emerged Aug. 11, 1922; adult still living.

TABLE I.—*Oviposition records of Tenebroides mauritanicus*—Continued

Date.	Mean temperature.	Number of eggs laid by Beetle No.—											
		330	331	332	336	337	339	340	542	543	609	658	659
1922.	°F												
ug. 24	76	....	36	....	10	....	.....	.....	8	15	.....	.....	.....
26	79	....	59	6	....	32	.....	.....	26	....	18	15	.....
27	79	....	.....	....	38	.....	.....	.....	.....	.....	.....	.....	14
28	70	18	.....	.....	.....	19	....	36	.....	.....	.....	.....	.....
29	75	....	.....	6	.....	.....	.....	.....	12	.....	.....	24	.....
30	74	....	.....	.....	.....	.....	.....	.....	12	....	20	.....	.....
31	76	....	3	....	25	.....	.....	.....	.....	.....	.....	.....	.....
ept. 4	79	....	.....	.....	9	.....	.....	.....	.....	.....	.....	.....	.....
5	80	....	8	....	.....	.....	.....	29	.....	.....	45	....	14
7	80	9	49	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	76	....	13	....	34	.....	.....	.....	.....	.....	.....	.....	.....
9	77	....	.....	.....	.....	.....	.....	.....	.....	.....	38	14	.....
10	78	....	.....	.....	23	.....	.....	.....	.....	.....	.....	.....	.....
12	77	....	25	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	73	11	.....	.....	.....	.....	.....	.....	.....	.....	.....	31	.....
14	74	....	16	....	15	.....	.....	.....	.....	.....	.....	.....	.....
16	78	....	43	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
17	70	....	.....	....	31	.....	.....	.....	.....	.....	.....	10	.....
18	62	....	10	.....	.....	.....	.....	.....	14	.....	.....	.....	18
19	68	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	6	.....
20	69	....	20	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
22	69	....	.....	....	10	.....	.....	.....	.....	.....	.....	.....	8
ct. 3	70	....	39	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	74	....	52	....	19	.....	.....	.....	.....	.....	.....	.....	.....
7	73	....	15	.....	.....	.....	.....	18	.....	.....	.....	.....	.....
10	73	....	23	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	68	....	27	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total to date	.....	530	1,190	301	541	436	176	387	244	88	138	100	54

Just how many eggs are normally laid by the female cadelle during her ifetime can not be stated exactly at this time. A large number of emale beetles were kept under observation during the season of 1922. A ortion of these emerged during the summer and had laid comparatively ew eggs before December. However, all of them were alive and hiber- ating at this time, and it was presumed that they would lay the bulk of heir eggs next season. The remainder of the beetles were collected in the early spring, so that their exact age is not known, but at the time of capture they were thought to have but recently emerged. The record of one of these latter beetles is thought worthy of special mention as indicating the remarkable egg-laying capacity of this species.

Female beetle No. 331 collected May 25, 1922 (its light color indicating that it had but recently emerged), laid its first eggs on July 1, 1922. At intervals varying from 1 to 5 days it continued to oviposit until September 20, 1922, when it stopped for a period of 13 days. On October 3, 1922, it started to lay eggs once more and continued oviposition at regular intervals until October 15, 1922. During the month of July it laid a total of 451 eggs, during August 399 eggs, during September 184, and during October 156 eggs, a grand total for the season of 1,190 eggs. The female died December 2, 1922.

Whether or not this number is greatly in excess of the average egg production of the species will be shown by further biological work now under way. Several of the other beetles collected in the spring laid more than 500 eggs during the season but none approached the record of No. 331.

INCUBATION PERIOD

The incubation period of the egg (see Table II) varies considerably, being influenced chiefly by the prevailing temperatures. During the latter part of April and the first week in May, when the temperature ranged between 54° and 78° F. with a mean of about 68°, the eggs almost invariably hatched in 10 days. As the weather became warmer the incubation period gradually decreased until during June, July, and August, when the temperature ranged between 70° and 90° F. with a mean of about 79°, only 7 days were required. With the return of cooler weather the period again lengthened, and in October, when the last eggs of the season were laid, 10 days were again required for incubation.

TABLE II.—*Life-history data of Tenebroides mauritanicus, Washington, D. C., 1922*

Number.	Date egg laid.	Date egg hatched.	Length of egg stage.	Date of first molt.	Length of first larval stage.	Date of second molt.	Length of second larval stage.	Date of third molt.	Length of third larval stage.	Date of fourth molt.	Length of fourth larval stage.	Date prepupal stage began.	Length of fifth larval stage.	Date of pupation.	Length of pre-pupal stage.	Date adult emerged.	Length of pupal stage.	Length of period from egg to adult.	Food of larva.
			Days.		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*	*Days.*	
36	Apr. 27	May 7	10	May 23	16	June 3	11	June 20	17	.....	25	July 15	.....	July 25	10	Aug. 7	13	102	Graham flour.
38	27	7	10	20	13	4	15	20	21	.....	25	15	.....	25	10	6	13	101	Do.
39	27	7	10	18	11	3	17	18	16	.....	23	23	.....	24	9	17	13	112	Do.
40	27	7	10	17	10	3	17	17	15	.....	27	19	.....	29	12	10	12	105	Do.
43	27	7	10	17	10	3	15	17	16	.....	19	15	.....	26	11	7	12	103	Do.
55	27	7	10	25	18	3	9	18	15	.....	17	Aug. 3	.....	Aug. 11	8	24	13	119	Corn.
58	27	7	10	23	15	7	12	20	15	.....	25	July 15	.....	July 25	10	7	13	102	Do.
66	27	7	10	23	15	6	16	23	17	.....	24	17	.....	24	7	3	10	98	Do.
70	May 5	15	10	28	13	7	9	21	15	July 20	29	28	8	Aug. 4	7	17	13	104	Do.
72	5	15	10	30	14	6	8	19	15	.....	26	15	.....	30	15	11	13	98	Do.
73	5	15	10	30	15	8	11	22	13	.....	23	15	.....	July 25	10	7	13	94	Do.
74	5	15	10	29	14	10	12	23	12	.....	27	19	.....	29	10	10	12	97	Do.
75	5	15	10	29	14	10	12	23	12	.....	25	15	.....	31	10	7	13	100	Do.
79	5	15	10	26	14	6	10	19	13	.....	32	21	.....	Aug. 1	11	13	12	104	Do.
82	5	15	10	27	11	9	14	20	16	.....	23	23	.....	3	14	14	13	104	Do.
130	5	15	10	27	11	10	15	20	17	.....	23	26	.....	7	16	17	13	126	Barley flour.
131	5	15	10	29	11	10	15	20	17	.....	21	Aug. 10	23	26	16	Sept. 8	13	113	Do.
135	5	15	10	27	11	10	15	20	17	.....	19	3	16	8	8	Aug. 26	15	98	Do.
64	6	15	9	May 27	12	11	14	21	17	July 6	22	July 16	.....	July 31	15	11	12	94	Corn.
68	6	15	9	28	13	10	9	21	14	.....	15	15	9	27	12	8	11	86	Do.
71	6	15	9	30	13	10	8	20	14	.....	14	15	.....	14	10	July 25	11	93	Wheat.
128	7	15	9	30	15	10	8	20	16	.....	19	18	.....	20	11	Aug. 8	11	93	Do.
164	7	16	9	31	15	11	8	18	12	July 11	23	17	.....	28	10	7	12	92	Do.
166	7	16	9	31	15	11	8	18	16	.....	16	21	.....	26	9	11	13	96	Do.
178	June 5	June 12	7	June 24	12	July 4	10	July 15	11	.....	14	29	.....	29	9	20	13	76	Corn.
181	5	12	7	24	12	5	11	17	12	.....	12	29	.....	29	12	23	13	78	Do.
182	5	12	7	23	10	5	11	16	13	.....	16	29	.....	29	9	19	13	75	Do.
192	5	12	7	23	10	5	11	16	13	.....	16	29	.....	29	9	20	13	75	Do.
209	6	13	7	25	12	5	10	16	11	.....	13	29	.....	29	9	19	13	74	Do.
210	6	13	7	25	12	6	11	19	13	.....	10	29	.....	29	9	19	13	74	Do.
218	7	13	6	26	9	5	9	18	13	.....	11	29	.....	29	9	20	13	76	Do.
247	11	17	6	26	9	5	8	17	13	.....	11	29	.....	29	9	19	13	78	Do.
257	11	17	6	29	9	5	6	17	13	.....	12	29	.....	29	9	19	12	72	Do.
309	16	23	7	2	9	13	11	24	11	.....	11	29	.....	29	11	27	12	72	Do.
317	19	26	7	July 7	11	19	12	28	9	.....	11	Aug. 4	.....	15	9	4	13	75	Do.
333	19	26	7	7	11	19	12	28	9	.....	11	8	.....	20	12	Sept. 2	10	73	Do.
304	July 3	July 10	7	7	10	19	12	28	16	.....	24	8	.....	20	12	Aug. 30	17	103	Do.
	3	10	7	20	10	31	12	28	16	.....	11	Sept. 9	.....	Sept. 27	18	Oct. 14	17	83	Do.
				20	10	Aug. 1	12	28	15	.....	13	Aug. 29	.....	10	13	Sept. 24	14		

TABLE II.—*Life-history data of Tenebroides mauritanicus, Washington, D. C., 1922*—Continued

Number.	Date egg laid.	Date egg hatched.	Length of egg stage.	Date of first molt.	Length of first larval stage.	Date of second molt.	Length of second larval stage.	Date of third molt.	Length of third larval stage.	Date of fourth molt.	Length of fourth larval stage.	Date prepupal stage began.	Length of fifth larval stage.	Date of pupation.	Length of pre-pupal stage.	Date adult emerged.	Length of pupal stage.	Length of period from egg to adult	Food of larva.
			Days.		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*	*Days.*	
371	July 3	July 10	7	July 20	10	July 31	11	Aug. 17	17	.....	17	Sept. 3	.....	Sept. 17	14	Oct. 1	14	90	Corn.
384	3	10	7	20	10	28	8		16	.....	20	2	.....	18	16	3	15	93	Do.
403	3	10	7	20	10	31	11	13	18	.....	23	4	.....	21	17	7	16	96	Do.
414	4	11	7	21	10	31	10	16	16	.....	19	4	.....	20	16	6	16	94	Do.
561	Aug. 3	Aug. 11	8	Aug. 23	12	Sept. 4	12	Sept. 20	16	(a)	.....	.....	.....	.....	.....	.....	.....	.....	Do.
562	3	11	8	26	15	6	11	26	14	(b)	.....	.....	.....	.....	.....	.....	.....	.....	Do.
573	7	14	7	24	10	11	11	26	29	Oct. 11	15	(c)	.....	.....	.....	.....	.....	.....	Do.
593	7	14	7	24	10	11	4	26	15	(d)	.....	.....	.....	.....	.....	.....	.....	.....	Do.
633	15	25	7	Sept. 7	13	20	9	Oct. 19	23	(e)	.....	.....	.....	.....	.....	.....	.....	.....	Do.
634	18	25	7	7	13	21	14	14	18	(f)	.....	.....	.....	.....	.....	.....	.....	.....	Do.
639	18	25	7	6	12	22	16	10	18	(g)	.....	.....	.....	.....	.....	.....	.....	.....	Do.
654	18	25	7	6	12	23	17	10	26	(h)	.....	.....	.....	.....	.....	.....	.....	.....	Do.

a Went into hibernation October 10, 1922.
b Went into hibernation October 12, 1922.
c Went into hibernation October 25, 1922.
d Went into hibernation October 19, 1922.
e Went into hibernation November 2, 1922.
f Went into hibernation October 27, 1922.
g Went into hibernation October 25, 1922.
h Went into hibernation November 1, 1922.

LENGTH OF LARVAL STAGE

Under favorable conditions and with the right kind of food the larval growth is very rapid. The shortest larval period observed was 39 days, from the date of hatching to the formation of the pupal cell. (See Table II.) This larva hatched on June 20, 1922, and was fed on corn. This case was not unusual, as many more individuals had larval periods of about the same length. Larvæ that hatch during the late summer or early fall do not transform that season but enter into hibernation and complete their growth the following spring.

Unfavorable conditions and improper food will lengthen the larval stage almost indefinitely. The larva will feed on an almost endless variety of foodstuffs but does not thrive equally well on all of them. Several hundred larvæ were reared in the laboratory under conditions that were identical except in the matter of food. It was found that the larvæ that were fed upon corn, wheat, and Graham flour grew rapidly, and completed their growth in approximately the same length of time, about 69 days. Larvæ fed upon barley flour did not grow quite so rapidly, but a few of them completed their growth about two weeks after the corn-wheat-Graham flour-fed larvæ. Larvæ fed upon rough rice grew still more slowly, and although apparently full grown at this time, six months after birth, none had transformed and it was presumed that they would not do so before the spring of 1923. Larvæ fed upon refined white flour were still quite small after six months of feeding and, judging from the present slow rate of growth, would have a very long larval life.

The foregoing observations may in part explain the phenomenally long larval period reported, first by Kirkup [1] in 1812 and recently by McColloch [2] in 1922. The single larva observed by Kirkup lived for more than 15 months without transforming, and larvæ observed by McColloch lived from 628 to 1,248 days before transforming.

The cadelle larvæ are generally believed to be carnivorous. The writer has found that they rarely molest any other insect larva and apparently never feed on the bodies of dead larvæ.

NUMBER OF LARVAL MOLTS

The cadelle larva as a rule molts either three or four times, although when for some reason or other the larval period is prolonged beyond its normal length many more molts may occur. (See Table II.) McColloch observed one larva to molt no less than 11 times.

Observations made show that larvæ that hatch in early summer may molt four times but usually molt but three times. Those hatching later in the season almost invariably molt four times. Data showing the number of molts and the length of time between molts are given in Table II.

PUPATION

After attaining its growth the larva becomes restless and wanders off to find a place of safety in which to transform. It prefers to burrow into a piece of soft wood, hollow out a small chamber, and close up the open end with cement made from the larval borings mixed with a larval secretion. The walls and floors of many wooden bins used to hold wheat

[1] KIRKUP, JOS. ACCOUNT OF TENEBRIO MAURITANICUS. *In* Trans. Ent. Soc. London, 1812, p. 329-331.

[2] MCCOLLOCH, J. W. LONGEVITY OF THE LARVAL STAGE OF THE CADELLE. *In* Jour. Ec. p. 242. 1922.

show evidence of their burrowing. The larva often crawls between two boards and forms its pupal cell between them, or it may form its cell in a hollowed-out kernel of corn. Larvæ bred in the laboratory were supplied in most cases with small pieces of cork, and when ready to transform they bored into the cork and soon disappeared from sight. The pupal form was assumed about nine days after the formation of the pupal cell.

The pupal stage lasted from 10 to 15 days in summer with an average of from 12 to 13 days. (See Table II.) Apparently the cadelle does not overwinter in the pupal stage.

LIFE CYCLE

It has been assumed by previous writers that there is but one generation of the cadelle each year. It appears from the investigations carried on at Washington, D. C., that the normal life cycle is as follows: Adults, and larvæ of all stages, pass the winter in hibernation. The overwintering adults lay eggs in the spring that hatch and develop through to adults by midsummer. These midsummer adults lay eggs, the larvæ from which overwinter in all stages of maturity. Many become full grown by fall, but probably none transform until the following spring. The larvæ that overwinter transform in the spring and the emerging beetles lay eggs all through the summer and hibernate during the following winter. This life cycle applies to the vicinity of Washington, D. C. Farther south, in subtropical and tropical climates, it seems probable that development would be more or less continuous and that there would be several generations a year.

CHEMICAL EXAMINATION OF "CHUFA," THE TUBERS OF CYPERUS ESCULENTUS LINNÉ[1]

By FREDERICK B. POWER, *Chemist in Charge*, and VICTOR K. CHESNUT, *Assistant Chemist, Phytochemical Laboratory, Bureau of Chemistry, United States Department of Agriculture*

HISTORICAL

Cyperus esculentus Linné, belonging to the family of *Cyperaecae*, is a native of southern Europe and North and South Africa. It is said to have been cultivated from the earliest times in Italy and North Africa on account of its edible tubers, which are commonly known under the Spanish name of *chufa*, but also as "earth almond" and "rush nut," and in French as *souchet comestible* or *amande de terre*.

According to a notice in the American Agriculturist (*1*),[2] the chufa was originally introduced into this country many decades ago as a food for swine, although it never fairly established itself as such, and, notwithstanding the fact that interest in the plant has from time to time been revived, it does not seem to be more generally cultivated than it was many years ago. The yield of chufa is said to be about 200 bushels to the acre, although some reports have indicated it to be very much larger. It was also noted in the above-mentioned publication that in some countries the tubers, which have a flavor somewhat like that of almonds, are expressed for their oil, of which they yield about 16 per cent. In Italy and Egypt, in fact, the fatty oil appears to have been used for the same purposes as olive oil, both as a food and for the manufacture of soap.

In addition to the use of Cyperus tubers in the south of Europe as an article of food, it is stated that when roasted and ground they may serve as a substitute for coffee and cocoa (*5*). Such a preparation was known in Germany many years ago as *mandelkaffee* ("almond coffee"), although many other coffee substitutes were subsequently sold under this name (*7*). We have ascertained that chufa contains no trace of caffeine, the presence of which was indeed not at all probable.

Some interesting information relating to the use of chufa in Spain was kindly brought to our notice by Dr. David Fairchild, of the Bureau of Plant Industry, United States Department of Agriculture. This is contained in an illustrated article entitled "Horchata de Chufas," which was published in the magazine *Blanco y Negro* at Madrid in 1901. In this article (*2*) it is stated that the summer beverage known as *horchata de chufa*,[3] of which large quantities are consumed, constitutes an industry of such importance that thousands of persons gain their living thereby in those regions where the material for its manufacture is cultivated and collected, and that a large number of establishments of all classes and grades are devoted to its exploitation. Besides the cafés and the *chuferia*

[1] Accepted for publication Aug. 11, 1923.

[2] Reference is made by number (italic) to "Literature cited," p. 75.

[3] The Spanish word *horchata* means literally an emulsion, and the beverage has been described as a kind of orgeat produced from chufa or Cyperus tubers. The term *orgeat*, derived from the French word for barley, *orge*, was originally given to a drink made from barley, but has since been applied to a beverage made from almonds, orange-flower water, and sugar.

—the latter name being given to the places where the *horchata* is made and sold—it is said that there exist in the streets of Madrid about three hundred *horchata* stands and many ambulatory salesmen of the same article.[4] The chufa is gathered on the shores of the river Segura, and the majority of persons engaged in the industry in any of its distinct branches are inhabitants of Valencia and Alicante. For the preparation of the beverage the Cyperus tubers are first washed in cylinders of wire netting by means of a continuous flow of water, then subjected to pressure, and the expressed milky liquid, which apparently contains the fatty oil in an emulsified state, is finally cooled or frozen.

The tubers of *Cyperus esculentus* appear to have been first qualitatively examined in 1822 by Lesant (*4*), who called attention to the importance of their cultivation in certain parts of France. A more complete investigation of them was recorded in 1851 by Luna (*6*), who noted the presence of 28 per cent of fatty oil (about 17 per cent by expression and 11 per cent by subsequent extraction with ether), 14 per cent of cane sugar, and 29 per cent of starch, together with small amounts of albumin, gum, and coloring matter. The oil was described as liquid at ordinary temperatures, but solidifying at 0° C., having the yellow color of olive oil, transparent and inodorous. Its density was found to be 0.9190 at 12° C., and the presence of olein was determined. The characters of the starch grains were also noted by Luna, but they have been much more completely described by Vogl (*7*).

Hell and Twerdomedoff (*3*), by extracting the tubers with light petroleum, obtained an average of 27.1 per cent of fatty oil, which was stated to have a yellow color and a not unpleasant odor, suggestive somewhat of burnt sugar, but its physical characters were not further described. They considered the oil to consist chiefly of olein with a little myristin.

Inasmuch as all the previous examinations of chufa appear to have been conducted with material obtained from southern Europe, it has seemed desirable that an investigation should be made of the tubers grown in this country. One of the most important constituents of the tubers is the fatty oil, and it was therefore of particular interest that this should be more completely examined.

PRELIMINARY TESTS

The material first examined by us was kindly supplied by Dr. David Fairchild, from the 1917 crop, purchased in Richmond, Va. This material consisted of the dried Cyperus tubers, which were pale brown in color, much wrinkled, and very small, their average weight being about 0.25 gm.

A small portion of the material (20 gm.) was tested for the presence of an alkaloid, but with a perfectly negative result.

Twenty-five gm. of the ground tubers were extracted successively in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100° C., were obtained:

Light petroleum (b. p. 35–55° C.) extracted	4.44 grams	=17.76 per cent.
Ether extracted	0.22 grams	= 0.88 per cent.
Chloroform extracted	0.05 grams	= 0.20 per cent.
Alcohol extracted	5.82 grams	=23.28 per cent.
Total	10.53 grams	=42.12 per cent.

[4] It is of interest to note incidentally the observation of Luna (*6*), in 1850, that in Madrid alone 12,000 kgm. of chufa were annually consumed in the preparation of orgeat, but he considered it more important that the tubers should be used for obtaining therefrom the oil, sugar, and starch.

The petroleum extract was a pale yellow, inodorous, fatty oil, and the ther extract consisted of a fatty substance. The chloroform extract, hich was very small in amount, was completely amorphous. The lcohol extract, after the removal of the solvent, formed a pale yellow, iscid sirup, having an agreeable, sweetish odor and taste. It readily educed Fehling's solution on heating, and evidently consisted chiefly f sugar.

For the purpose of a more complete examination a larger quantity of naterial was subsequently employed.

THE FATTY OIL

Fifteen kgm. of the ground chufa were extracted as completely as ossible with cold light petroleum (b. p. 35–55° C.). After the removal f the solvent, which was finally effected by heating on a steam bath in current of carbon dioxid, the amount of fatty oil obtained was 4345 m., or 28.9 per cent. The yield of oil, as recorded in the literature, ppears to be subject to considerable variation, which doubtless depends argely on the amount of moisture contained in the tubers, and also on he method of extraction. The smaller amount of material with which ur preliminary experiments were conducted yielded by extraction with ight petroleum 18.2 per cent of oil.

Chufa oil has a pale yellow or reddish-yellow color and very little dor or taste. The characteristics of the product obtained by us were determined in the Oil, Fat, and Wax Laboratory of the Bureau of Chemistry by W. F. Baughman, and for this purpose the oil was deprived of the last traces of petroleum by heating it under diminished pressure at a temperature of 145–150° C. in a current of carbon dioxid. The following results were obtained:

Specific gravity 25°/25°	0.9120
Refractive index at 20°	1.468
Iodin value (Hanus)	76.6
Saponification value	191.5
Acid value	15.7

A complete chemical examination of the oil has now also been made by Baughman and Jamieson, the results of which are embodied in a separate communication to this number of the Journal of Agricultural Research (p. 77).

EXTRACTION OF THE CHUFA WITH ALCOHOL

ISOLATION OF SUCROSE

Two kgm. of the material from which the fatty oil had been removed by means of light petroleum were completely extracted with hot alcohol. After the evaporation of the greater part of the solvent a reddish-yellow liquid was obtained from which a considerable quantity of crystals was soon deposited. These crystals, which evidently consisted of sucrose, were collected, dissolved in water, and an attempt made to extract the liquid with ether, but a jelly was thus formed and no separation could be effected. The ether was subsequently removed, and to the turbid aqueous liquid, which did not permit of filtration, a slight excess of a solution of basic lead acetate was added. A small amount of precipitate was thus produced, and the mixture acquired a pale reddish or fawn color. After filtration by suction, and the removal of the lead by hydrogen sulphid, a perfectly colorless liquid was obtained. This was extracted

several times with ether, and the colorless ethereal liquids, after being washed with a little water, were added to the ether extract from the main portion of the original liquid. On finally concentrating the aqueous liquid there was obtained a quantity (25 gm.) of nearly colorless crystals, which were further purified by treatment with a little animal charcoal and recrystallization. The crystals, after being dried in a desiccator, yielded a solution which did not reduce Fehling's solution until heated with an acid, and they possessed all the other characters of sucrose. A determination of the specific optical rotation gave the following result:

0.6312 gm. dissolved in water to the measure of 25 cc. at 20° C. gave $+3.33°$ in a 2 dcm. tube, whence $[\alpha]_D = +65.9°$.

These figures are in close agreement with those required for sucrose, which has $[\alpha]_D + 66.5°$.

The main portion of the alcoholic extract, from which the above-described crystals of sucrose had separated and from which the alcohol had been removed, gave no precipitate on further dilution with water, and consequently no resinous material was present. On the subsequent addition of a solution of basic lead acetate an abundant brick-red precipitate was produced. This was separated by filtration and well washed with hot water.

BASIC LEAD ACETATE PRECIPITATE

The material precipitated by basic lead acetate, including the small amount previously obtained by the purification of the sugar solution, was mixed with water and decomposed by hydrogen sulphid. The filtrate from the lead sulphid was concentrated, when it formed a reddish-yellow liquid. It was extracted several times with ether, but this removed only a small amount of amorphous material, which, when dissolved in water, gave a grayish-brown coloration with ferric chlorid, thus indicating the possible presence of a little tannin. After extraction with ether, the liquid abundantly reduced Fehling's solution, and evidently contained considerable sugar which had been occluded in the original lead precipitate.

FILTRATE FROM THE BASIC LEAD ACETATE PRECIPITATE

This liquid was first treated with hydrogen sulphid for the removal of the excess of lead, the mixture filtered, and a current of air passed through the filtrate to expel the dissolved gas. The liquid then possessed only a faintly yellow color. It was extracted several times with ether, but only a very small amount of amorphous material was thus removed. After concentrating the liquid it formed a viscid, reddish-yellow sirup which gave no reaction with the ordinary alkaloidal reagents or with a solution of mercuric nitrate. It readily reduced Fehling's solution and on heating with a caustic alkali developed ammonia. A small portion of the sirup yielded an abundance of *d*-phenylglucosazone, which on crystallization from 60 per cent alcohol separated in fine yellow needles, melting and decomposing at 217–218° C. It therefore consisted chiefly of a reducing sugar.

EXTRACTION OF THE CHUFA WITH WATER

SEPARATION OF STARCH AND EVIDENCE OF THE PRESENCE OF AN ENZYM

One hundred gm. of the ground tubers were mixed with 1000 cc. of water and allowed to stand over night. The mixture was then strained through a fine cloth, and the deposited starch collected and washed,

when, after drying, it amounted to 12 gm., or 12 per cent of the weight of the tubers.[5] This starch formed a perfectly white powder, and its very dilute aqueous solution gave a bright blue color with iodin.

The clear, aqueous filtrate from the starch was mixed with twice its volume of alcohol, when a very slight flocculent precipitate was produced. After standing for several hours the precipitate was collected, washed with a little alcohol, and dried. The dark-colored product could then be triturated to a brownish powder, and amounted to 0.6 gm. Its aqueous solution gave the biuret reaction, a precipitate with potassium-mercuric iodid, and developed ammonia on heating with a caustic alkali, thus showing the characters of a protein. It also slowly hydrolyzed amygdalin, which indicated the presence of an enzym.

Two kgm. of the chufa, consisting of material from which the fatty oil had previously been extracted with light petroleum, were mixed with 8 liters of water, and the mixture allowed to stand for two days. It was then strained, the expressed marc mixed twice successively with two portions of water of 4 liters each, and each time strained as before. The deposited starch was collected, washed first by affusion and decantation with water, subsequently on a filter with alcohol, and finally dried. It was thus obtained as a nearly white powder and amounted to 360 gm. Inasmuch as the 2 kgm. of chufa deprived of oil represented 2813 gm. of the original tubers, the calculated yield of starch would be 12.8 per cent, or only a little more than was obtained in the previously described experiment.

The clear aqueous filtrate from the starch was treated with a slight excess of a solution of basic lead acetate, which caused a voluminous grayish precipitate.

BASIC LEAD ACETATE PRECIPITATE

This material, after being washed with water, was suspended in water, decomposed by hydrogen sulphid, and the mixture filtered. The filtrate from the lead sulphid had a pale yellow color and was concentrated under diminished pressure to a small volume. On the addition of alcohol it gave a considerable precipitate of gum, which was removed by filtration, and after further purification was obtained as a nearly white powder. After the removal of the alcohol the dark colored aqueous liquid had a strongly acid reaction, gave no coloration with ferric chlorid, but abundantly reduced Fehling's solution, which was evidently due to the occlusion of sugar by the original lead precipitate. The liquid was extracted several times with ether, which, however, removed only a very small amount of material containing nothing of interest. As nothing could be obtained directly from the liquid it was finally divided into two equal portions, one of which was heated with 5 per cent of its weight of sulphuric acid and the mixture extracted with ether, while the other portion was heated with an amount of potassium hydroxid corresponding to 10 per cent of its weight. This alkaline mixture, after being acidified with sulphuric acid, was likewise extracted several times with ether. In both cases only a very small amount of dark colored, amorphous material was obtained.

[5] The amount of starch actually present in the tubers is somewhat greater than that obtained, but its exact quantitative determination was not attempted. Luna (6) has recorded the presence of 29 per cent of starch in the European chufa, but his determination was made by polarimetric observations after hydrolysis of the starch and a calculation based on the amount of cane sugar present, which was also determined polarimetrically before inversion. As the optical rotations would also have been influenced by any reducing sugar originally contained in the liquid, which was not directly determined, there would appear to be a possibility of error.

FILTRATE FROM THE BASIC LEAD ACETATE PRECIPITATE

This liquid was first treated with hydrogen sulphid for the removal of the excess of lead and the mixture filtered. The filtrate was then concentrated under diminished pressure to the consistency of a syrup, when it had a reddish-brown color. It was extracted several times with ether, but nothing of interest was removed. On heating a little of the syrup with a caustic alkali it developed ammonia, and it abundantly reduced Fehling's solution, thus showing the presence of considerable sugar. By repeated treatment of the syrup with alcohol a small portion was finally obtained which was soluble in nearly absolute alcohol. This was tested in the usual manner for the presence of the simple organic bases, such as cholin and betain, but with a negative result. The portion of the syrup which remained undissolved by the treatment with alcohol was examined for asparagin, which was found not to be present. The chief constituent of the syrupy liquid was evidently a reducing sugar.

SUMMARY

The tubers of *Cyperus esculentus*, Linné, commonly known by the Spanish name of "chufa," were chemically examined many years ago, but all the material employed for this purpose was evidently obtained from southern Europe. It therefore appeared of interest to ascertain the constituents of the tubers grown in this country, and it has been found that these are essentially the same as those recorded by previous investigators.

The most important constituent of the chufa is unquestionably the fatty oil, which, as extracted by light petroleum, was obtained by us in one instance to the extent of 28.9 per cent. Although the yield of oil is subject to considerable variation, being chiefly influenced by the amount of moisture contained in the tubers, it is nevertheless very remarkable that so large a proportion of such a product should be contained in the underground portion of a plant, and particularly of a sedge. A complete investigation of the fatty oil prepared by us has been made in the Oil, Fat and Wax Laboratory of the Bureau of Chemistry by Baughman and Jamieson, whose results are recorded in a separate paper in this number of the Journal of Agricultural Research (p. 77).

Other constituents of the chufa which would appear to be of economic interest are sucrose and starch, both of which are present in considerable amounts. The sucrose or cane sugar has been obtained in a pure crystalline state, but it is accompanied in the tubers by a reducing sugar, together with gummy and albuminous material, which renders its separation somewhat difficult.

The starch was obtained in the form of a perfectly white powder. When separated directly from the chufa the yield was 12 per cent, whereas from material which previously had been deprived of the fatty oil it was 12.8 per cent. The gum, which is present in relatively small proportion, is precipitated by basic lead acetate. After purification it was obtained as a nearly colorless powder. A constituent of the tubers which has not been recorded hitherto is an enzym. This is present in only very small amount, and is capable of slowly hydrolyzing amygdalin.

The tubers did not respond to the general tests for an alkaloid, and were found to contain no caffeine or asparagin. There was also no indication of the presence of such widely distributed simple organic bases as cholin and betain.

Although the chufa is a well known agricultural product, the character its constituents would suggest the possibility of a more extended lization than is at present the case.

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HE CONSTITUENTS OF "CHUFA" OIL, A FATTY OIL FROM THE TUBERS OF CYPERUS ESCULENTUS LINNÉ[1]

y WALTER F. BAUGHMAN, *Associate Chemist*, and GEORGE S. JAMIESON, *Chemist in Charge, Oil, Fat and Wax Laboratory, Bureau of Chemistry, United States Department of Agriculture*

A chemical examination of the constituents of chufa has been made y Power and Chesnut, and the results obtained by them are embodied a separate communication to this number of the Journal of Agricul- ral Research (p. 69). The fatty oil obtained by these investigators as submitted to this laboratory for a more complete determination of s constituents.

Chufa oil, which is sometimes known as sedge oil, and in Germany as rth almond oil (*Erdmandeloel*), has received occasional mention in emical literature for a great many years, but has never been given a ry complete examination. As early as 1851 R. T. M. Luna published a aper relating to the tubers of this plant, in which he described a few xperiments he had made with the oil (7).[2] Carl Hell and S. Twerdome- off attempted to determine, in a qualitative way, the constitutents of e oil, and reported it (*4*) to consist principally of olein with a smaller mount of myristin, but they were not able to ascertain the presence f higher fatty acid glycerids. As will be shown later, the present uthors have verified the presence of a large percentage of oleic acid, ut have detected only a trace of myristic acid (less than 0.01 per cent), nd are unable to explain Hell and Twerdomedoff's apparently good vidence of its presence in much larger amounts, although the oil em- loyed by them was extracted from tubers grown in Europe.

The oil used for this investigation was extracted from the ground ubers with petroleum ether. It was not put through any refining process nd, therefore, should not be considered as consisting of pure fatty acid lycerids, but, like other crude oils, should be expected to contain more r less nonfatty material.

CHEMICAL AND PHYSICAL CHARACTERISTICS

The more important data are as follows:

pecific gravity $25°/25°$	0.9120	
efractive index, 20°	1.4680	
odin number (Hanus)	76.5	
aponification value	191.5	
Insaponifiable matter (per cent)	0.6	
cid value	15.7	
cetyl value	10.5	
eichert-Meissl number	0.2	
olenske number	0.3	
aturated acids, per cent (observed)	18.3	(Iodin number 6.5)
Insaturated acids, per cent (observed)	74.6	
aturated acids, per cent (corrected)	17.1	
Insaturated acids, per cent (corrected)	75.8	
odin number of unsaturated acids	96.9	

[1] Accepted for publication Aug. 11, 1923.
[2] Reference is made by number (italic) to "Literature cited," p. 82.

The low iodin number places this oil in the nondrying class. Th acid value is high for a fresh oil extracted from sound material, and thi is probably due to a very active fat-splitting enzym in the tuber. Th percentages of saturated and unsaturated acids were determined by th lead-salt-ether method and corrections made for the small amount o the unsaturated acid fraction which is weighed with the saturated aci fraction (*1*, *3*). The sum of the percentages of saturated and unsat urated acids is 92.9, instead of approximately 95.0, which would hav been expected if the oil had been refined. The low summation is due t the presence in the oil of nonfatty material as mentioned above. Th low Reichert-Meissl and Polenske numbers indicate the presence of onl a trace of volatile fatty acids.

UNSATURATED ACIDS

The iodin number of the unsaturated acid fraction (96.9) indicate that it consists almost completely of oleic acid. The bromin additio derivatives of the unsaturated acids were prepared (*6*, *p.* 579). N hexa-bromid, the derivative of linolenic acid, was found, but a sma amount of linolic tetrabromid, m. p. 114.0, was obtained. Using th iodin number of the unsaturated acid fraction and the theoretica iodin numbers of oleic acid (90.1) and linolic acid (181.4), the percentag composition of the unsaturated acid fraction was calculated to be 7. per cent linolic acid and 92.6 per cent oleic acid, or 5.9 per cent linoli acid glycerid and 73.3 per cent oleic acid glycerid in the original oil.

SATURATED ACIDS.

A quantity of saturated acids prepared by the lead-salt-ether metho was esterified with methyl alcohol (*5*). This mixture of methyl ester was then fractionally distilled under diminished pressure. The data fo this distillation are given in Table I. The mixture was divided int five fractions, designated by the letters A to E, and a residue by a pre liminary distillation from a 1-liter Claisen flask. These preliminar fractions were redistilled from a 250-cc. Ladenburg flask, as indicate in the table, and eight fractions and a residue obtained.

The iodin numbers, which are a measure of the contaminating unsat urated acids, and the saponification values of these fractions, are give in columns 2 and 3 of Table II. From the iodin numbers of thes fractions the percentage of unsaturated acids in each fraction wa calculated, and from these data the mean molecular weights of th saturated acids in each fraction were calculated as given in column 6 (*2*)

TABLE I.—*Fractional distillation of methyl esters of saturated acids*

[97.8 gm. subjected to distillation]

Fraction.		Temperature.	Pressure.	Weight.
		°C.	Mm.	Gm.
.....		179–184	9	23.00
.....		185–186	9	23.00
.....		186–189	9	22.15
).....		189–197	9	17.70
.....		198–208	9	6.15
tesidue.....		.....	.....	5.58
				97.58
raction A and B distilled.....	1	144–148	2	5.00
	2	148–151	2	24.70
raction C added.....	3	152–154	2	21.55
raction D added.....	4	154–159	2	14.57
raction E added.....	5	162–165	2.5	18.11
tesidue added.....	6	166–176	2.5	8.90
	7	177–210	2.5	2.85
	8	210–230	2.5	1.70
tesidue.....		.....	.....	.20
				97.58

TABLE II.—*Results of analyses of fractions obtained by distilling methyl esters of saturated acids*

Fractions.	Iodin number.	Saponification value.	Mean molecular weight.	Esters of unsaturated acids.	Mean molecular weight of esters of saturated acids.	Myristic acid.		Palmitic acid.		Stearic acid.		Arachidic acid.		Lignoceric acid.	
				Per cent.		*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*
1	3.2	207.0	271.0	3.45	270.1	0.65	0.03	90.89	4.54	.....	.....	.....	.....	.....	.....
2	3.7	205.9	272.5	3.99	271.7	.....	.....	86.50	21.37	4.56	1.13	.....	.....	.....	.....
3	6.9	202.6	276.9	7.44	275.4	.....	.....	71.84	15.48	16.01	3.45	.....	.....	.....	.....
4	12.6	198.2	283.0	13.60	281.2	.....	.....	50.15	7.31	31.94	4.65	.....	.....	.....	.....
5	15.7	195.1	287.5	16.94	285.9	.....	.....	35.04	6.35	43.95	7.96	.....	.....	.....	.....
6	17.6	188.0	298.4	18.99	299.0	.....	.....	.....	.....	75.56	6.72	1.66	0.15	.....	.....
7	9.7	175.3	320.0	10.46	323.0	.....	.....	.....	.....	10.36	0.30	75.30	2.15	.....	.....
8	7.2	155.4	361.0	7.77	367.6	.....	.....	.....	.....	.....	.....	23.45	0.40	65.25	1.11
															0.14
							0.03		55.05		24.21		2.70		1.25

The results in column 6 indicate what saturated acids may be present in the various fractions. The mean molecular weight of the saturated acid esters in fraction 1 is slightly below that of methyl palmitate (270.3), and indicates that the fraction is methyl palmitate with a trace of methyl myristate. The mean molecular weights of the saturated acid esters of fractions 2 to 5 lie between the molecular weights of methyl palmitate and methyl stearate (298.4), which indicates that these four fractions contain these two esters in various proportions. The probable constituents of fractions 6 and 7 are methyl stearate and methyl arachidate (326.4), and those of fraction 8 methyl arachidate and methyl lignocerate (328.5).

The free acids were recovered from some of these fractions and the residue by saponifying with alcoholic potash and decomposing the resulting soap with hydrochloric acid. The consituent acids were then isolated by fractional crystallization from alcohol. Their identity was established by the melting points and by observing whether or not these melting points were lowered when the substances were mixed with equal amounts of the respective acids which they were suspected of being, and the purity of which had been established previously by elementary analyses.

In all cases the melting points of the isolated acids confirmed deductions drawn from the mean molecular weights of the fractions. The following acids were isolated:

Lignoceric acid, $C_{24}H_{48}O_2$. From the residue 0.14 gm. of fatty acid melting at 80.5° was obtained. It was also identified in fraction 8.

Arachidic acid, $C_{20}H_{40}O_2$. This acid was identified in fractions 6 and 7 by the melting point 76.5°.

Stearic acid, $C_{18}H_{36}O_2$. Isolated from fractions 4, 5, and 6 and identified by the melting point 68–69°.

Palmitic acid, $C_{16}H_{32}O_2$. Its presence was proven in fractions 1, 2, and 3 by the melting point 63°.

Myristic acid, $C_{14}H_{28}O_2$. Eight subfractions were obtained from fraction 1 by its fractional crystallization from alcohol. The first five subfractions melted at 63°, the sixth and seventh at 60–61°, and the eighth at 54–55°. This eighth subfraction was mixed with an equal quantity of myristic acid of known purity and the melting point of the mixture taken. It was found to be 54–55°, which proved the eighth subfraction to be myristic acid.

The identity of the saturated acids in the various fractions and the residue obtained by distillation having been established, the quantities were calculated from the mean molecular weights of the saturated acid esters (column 6, Table II) and the theoretical molecular weights of the two esters in each fraction. The results are given in columns 7–16, Table II.

TABLE III.—*Saturated acids*

	Acids in saturated acid fraction.		Acids in original oil.	Glycerids in original oil.
	Gm.	*Per cent.*	*Per cent.*	*Per cent.*
Myristic	0.03	0.04	Trace.	Trace.
Palmitic	55.05	66.14	11.3	11.8
Stearic	24.21	29.08	5.0	5.2
Arachidic	2.70	3.24	.5	.5
Lignoceric	1.25	1.50	.3	.3
	83.24	100.00	17.1	17.8

In Table III the percentage composition of the saturated acid fraction is given in column 2, the percentages of saturated acids in the original oil in column 3, and the equivalent percentages of glycerids in column 4.

THE UNSAPONIFIABLE CONSTITUENTS OF THE OIL: ISOLATION OF A PHYTOSTEROL

The unsaturated acids separated from the oil contained the unsaponifiable constituents, and this material was submitted to Dr. Frederick B. Power, in charge of the Phytochemical Laboratory of the Bureau of Chemistry, for further investigation.

The method of procedure for this purpose consisted in combining the acids with potassium hydroxid, adding to the alkaline mixture a quantity of clean sea sand, and evaporating on a steam bath until a perfectly dry homogeneous product was obtained. This was extracted in a Soxhlet apparatus with light petroleum (b. p. 32–70° C.), which removed a small quantity (1.59 gm.) of somewhat impure crystalline material. After crystallization from warm alcohol the substance was obtained in the form of handsome colorless needles, melting at 134–135° C., which gave the characteristic color reactions of the phytosterols. A portion of the substance was treated with acetic anhydrid, when an acetyl derivative was obtained. The latter, when crystallized from warm alcohol, separated in fine, colorless needles, melting at 122–123°.

SUMMARY

The chemical composition of crude chufa oil has been determined with the following results:

Glycerid of myristic acid, trace.
Glycerid of palmitic acid, 11.8 per cent.
Glycerid of stearic acid, 5.2 per cent.
Glycerid of arachidic acid, 0.5 per cent.
Glycerid of lignoceric acid, 0.3 per cent.
Glycerid of oleic acid, 73.3 per cent.
Glycerid of linolic acid, 5.9 per cent.
Nonfatty material (by difference), 3.0 per cent.

A phytosterol (m. p. 134–135° C.) was isolated from the unsaponifiable material.

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